Bioorganic & Medicinal Chemistry 20 (2012) 2067–2081

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and pharmacology of 1-alkyl-3-(1-naphthoyl)indoles: Steric and electronic effects of 4- and 8-halogenated naphthoyl substituents

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ARTICLE INFO

Article history:
Received 8 December 2011
Revised 20 January 2012
Accepted 22 January 2012
Available online 30 January 2012

Keywords:
Cannabinoids
Structure–activity relationships
Cannabinoid receptors
Aminoalkylindole

ABSTRACT

To develop SAR at both the cannabinoid CB_1 and CB_2 receptors for 3-(1-naphthoyl)indoles bearing moderately electron withdrawing substituents at C-4 of the naphthoyl moiety, 1-propyl and 1-pentyl-3-(4-fluoro, chloro, bromo and iodo-1-naphthoyl) derivatives were prepared. To study the steric and electronic effects of substituents at the 8-position of the naphthoyl group, the 3-(4-chloro, bromo and iodo-1-naphthoyl)indoles were also synthesized. The affinities of both groups of compounds for the CB_1 and CB_2 receptors were determined and several of them were evaluated in vivo in the mouse. The effects of these substituents on receptor affinities and in vivo activity are discussed and structure–activity relationships are presented. Although many of these compounds are selective for the CB_2 receptor, only three JWH-423, 1-propyl-3-(4-iodo-1-naphthoyl)indole, JWH-422, 2-methyl-1-propyl-3-(4-iodo-1-naphthoyl)indole, the 2-methyl analog of JWH-423 and JWH-417, 1-pentyl-3-(8-iodo-1-naphthoyl)indole, possess the desirable combination of low CB_1 affinity and good CB_2 affinity.

1. Introduction

In the four decades since Gaoni and Mechoulam reported the elucidation of the structure of Δ^9-tetrahydrocannabinol (**1**, THC), the principal psychoactive constituent of marijuana,[1] comprehensive structure-activity relationships (SAR) have been developed based upon the dibenzopyran nucleus of THC.[2–6] The pharmacological effects of cannabinoids are considered to be mediated through at least two G-protein-coupled, transmembrane receptors. One of these, the CB_1 receptor, is found predominantly in the central nervous system and is thought to be responsible for most of the overt pharmacological effects of cannabinoids.[5,7–9] A second receptor, designated CB_2, was originally identified from macrophages present in the spleen, and is expressed primarily in the periphery.[10]

In 1991 a group at Sterling–Winthrop reported that pravadoline (**2**) and a number of other structurally similar indole derivatives inhibited contractions of the electrically stimulated mouse vas deferens.[11] Subsequent work revealed that these compounds also inhibit adenylate cyclase, are antinociceptive and interact with a G-coupled protein in the brain. It was also found that these aminoalkylindoles exhibit typical cannabinoid pharmacology in vivo and that the G-coupled protein with which they interact is the cannabinoid CB_1 receptor.[12,13] One of these aminoalkylindoles, WIN-55,212-2 (**3**), developed by the Winthrop group, is not only very potent in vivo, but has high affinity for both the cannabinoid CB_1 and CB_2 receptors.[14,15] The Winthrop group synthesized more than 100 aminoalkylindoles and proposed preliminary SAR[12,13,16] These SAR included the observation that a group larger than methyl at C-2 of the indole nucleus greatly attenuates potency and that a bicyclic aroyl group, usually 1-naphthoyl or a substituted 1-naphthoyl group, at C-3 of the indole is essential for potency. They also concluded that an aminoalkyl group, usually substituted aminoethyl, attached to the indole nitrogen was essential for cannabinoid activity.

In 1994 we reported that the aminoalkyl group appended to the indole nitrogen could be replaced by an alkyl group to provide relatively simple indole derivatives that exhibit typical cannabinoid pharmacology.[17] In particular, JWH-018, 1-pentyl-3-(1-naphthoyl)indole (**4**) has high affinity (K_i = 9 ± 5 nM) for the CB_1 receptor and exhibits typical cannabinoid pharmacology in vivo.[18] 1-propyl-2-methyl-3-(1-naphthoyl)indole JWH-015 (**5**) has relatively high affinity for the CB_2 receptor (K_i = 13.8 ± 4.6 nM), modest affinity for the CB_1 receptor (K_i = 164 ± 22 nM) and was one of the first compounds found to have useful selectivity for the CB_2 receptor.[15]

In subsequent work directed toward the development of improved CB_2 cannabinoid receptor ligands and to establish SAR for cannabimimetic indoles at both the CB_1 and CB_2 receptors, a number of additional indole derivatives were prepared and their pharmacology was evaluated.[19–21] It was found that CB_1 receptor affinity is optimal with a 1-pentyl nitrogen substituent and

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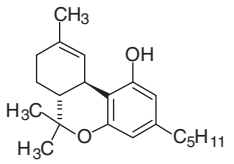
doi:10.1016/j.bmc.2012.01.038



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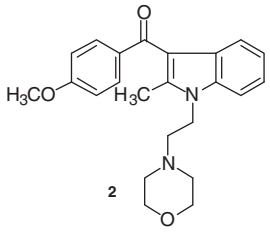

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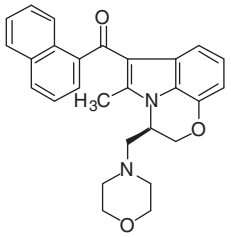

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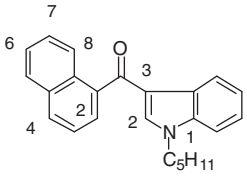

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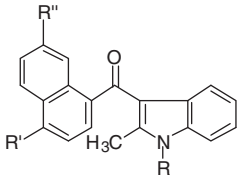
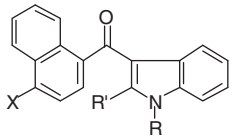
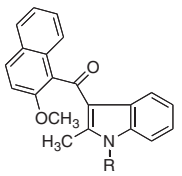


5 R = C_3H_7, R', R" = H
6 R = C_5H_{11}, R' = C_2H_5, R" = H
7 R = C_5H_{11}, R' = H, R" = CH_3
8 R = C_5H_{11}, R' = H, R" = C_2H_5

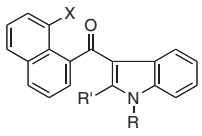


9 X = F
10 X = Cl
11 X = Br
12 X = I

decreases dramatically with *N*-alkyl substituents of three or less carbon atoms or more than six carbon atoms. In agreement with the observations of the Winthrop group, a 2-methyl substituent (for numbering see structure **4**) slightly decreases affinity at the CB_1 receptor, while larger substituents at this position decrease CB_1 receptor affinity considerably.[14,16,20] A small 4-alkyl-1-naphthoyl group at C-3 of the indole, as in JWH-213 (**6**) enhances both CB_1 (K_i = 1.5 ± 0.2 nM) and CB_2 receptor affinity (K_i =0.42 ± 0.05 nM), while a 7-methyl or ethyl-1-naphthoyl substituent as in JWH-048 (**7**) and JWH-262 (**8**) has relatively little effect on affinity for either receptor.[20,21] The effect upon CB_1 and CB_2 receptor affinity of methoxy substituents at C-2, C-4, C-6 and C-7 of the 1-naphthoyl moiety has also been described. A 2- or 6-methoxy-1-naphthoyl group greatly attenuates affinity for the CB_1 receptor, but has little effect upon CB_2 affinity. A 4-methoxy-1-naphthoyl group enhances affinity for both receptors while a 6-methoxy substituent reduces CB_1 receptor affinity significantly, but has only a slight effect upon CB_2 binding. A 7-methoxy group has relatively little effect upon affinity for either receptor.[20,21]



13 R = C_3H_7 or C_5H_{11}



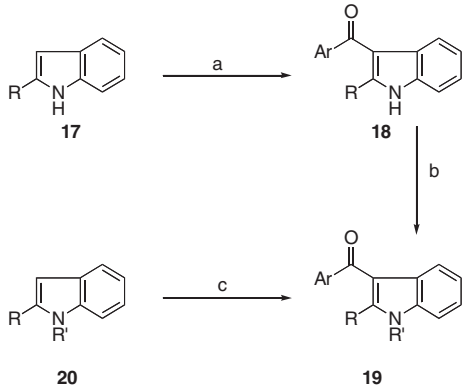
14 X = Cl
15 X = Br
16 X = I

These studies provide some indication of the effect of various naphthoyl substituents upon the CB_1 and CB_2 receptor affinities of 1-pentyl and 1-propyl-3-(1-naphthoyl)indoles. However, all of the substituents on the naphthoyl moiety examined to date are weakly to moderately electron releasing. In order to explore the effect upon receptor affinity of electron withdrawing substituents at C-4 of the naphthoyl, 1-propyl- and 1-pentyl-3-(1-naphthoyl-4-halo)indoles (**9**–**12**, R = C_3H_7 or C_5H_{11}, R′ = H or CH_3) were synthesized and their pharmacology was evaluated. In earlier work it was found that 1-propyl- and 1-pentyl-2-methyl-3-(2-methoxy-1-naphthoyl)indoles (**13**) had restricted rotation about the naphthalene C-1 bond to the carbonyl carbon, with a calculated energy barrier of 12.9 kcal/mol and an experimentally determined barrier of 13.1 kcal/mol.[20] In addition, two of the compounds in this series were very selective for the CB_2 receptor. It seemed probable that the 3-(8-halogenated-1-naphthoyl)indole derivatives (**14**–**16**, R = C_3H_7 or C_5H_{11}, R′ = H or CH_3) would exhibit similar restricted rotation, would be readily available and would provide a second series of indole derivatives with electron withdrawing groups on the naphthalene moiety, providing additional insight into the SAR of the cannabimimetic indoles. The choice of the *N*-propyl substituent is based on the observation that JWH-015 (**5**), a highly CB_2 selective indole derivative, contains this substitution pattern.[15,19] The *N*-pentyl group was chosen since this substituent almost invariably provides compounds with higher CB_1 receptor affinities than are observed with other nitrogen substituents.

2. Results

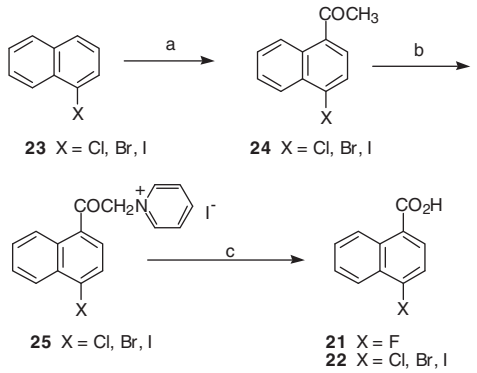
As shown in Scheme 1, two synthetic protocols have been developed for the preparation of various 1-alkyl-3-(1-naphthoyl)indoles. In one of the schemes, indole or 2-methylindole (**17**, R = H or CH_3) is reacted with methylmagnesium bromide and the resulting ambident anion upon reaction with the aroyl chloride provides 3-acylindole **18**.[11,17] Alkylation on nitrogen is effected using a primary alkyl halide with KOH in DMSO to afford the target 3-acylindole (**19**). Although this sequence is satisfactory and uses readily available aroyl chlorides, the yields in the first step are quite variable and intermediate **18** is difficult to purify. Alternatively, acylation of an N-substituted indole or 2-methylindole (**20**) using an acyl chloride and dimethylaluminum chloride provides the 3-acylindole in from fair to good yield.[20] In this procedure, which was developed by Okauchi, the indole is stirred with the Lewis acid for 30 min prior to addition of the acyl chloride.[22] Although this procedure appears to be a modified Friedel–Crafts reaction, there is evidence that it proceeds via a 3-indolylaluminum intermediate.[23] Unless otherwise noted, cannabimimetic indoles **9**–**12** and **14**–**16** were prepared via the Okauchi procedure.

For the synthesis of the 4-halogenated naphthoylindoles (**9**–**12**), 4-fluoro-1-naphthoic acid (**21**, Scheme 2) is the only 4-halo-1-naphthoic acid that is commercially available. 4-Chloro-, 4-bromo

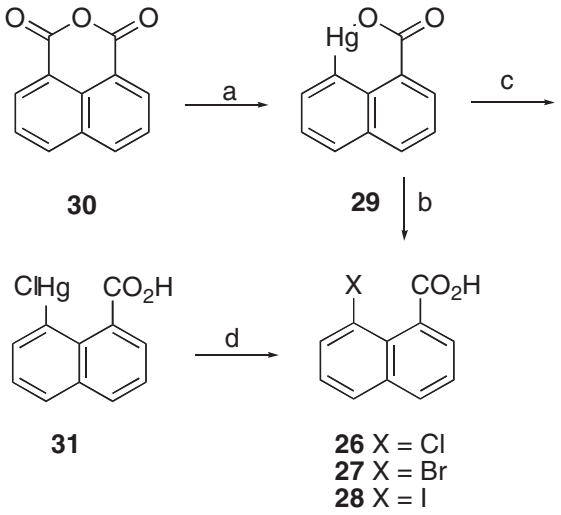


17 **18** **19** **20**

Scheme 1. Reagents and conditions: (a) MeMgBr, Et_2O/THF, 0 °C, then ArCOCl, reflux; (b) R′Br, KOH, DMSO, 80 °C; (c) Me_2AlCl, CH_2Cl_2, 30 min, 0 °C, then ArCOCl, 25 °C.



Scheme 2. Reagents and conditions: (a) CH_3COCl, $AlCl_3$, $ClCH_2CH_2Cl$, rt; (b) I_2, pyridine, 100 °C; (c) $NaOH/H_2O$, reflux, then HCl.



Scheme 3. Reagents and conditions: (a) NaOH, H_2O then HOAc, $Hg(OAc)_2$; (b) Br_2/NaBr or I_2/KI·HOAc; (c) 1 M $NaOH/H_2O$, NaCl then HCl; (d) Cl_2, HOAc.

and 4-iodo-1-naphthoic acids (**22**) were synthesized as shown in Scheme 2. Friedel-Crafts acylation of 1-chloro, 1-bromo or 1-iodonaphthalene (**23**) with acetyl chloride under standard reaction conditions provided the corresponding 4-halogenated-1-acetylnaphthalene (**24**). Reaction of ketones **24** with iodine and pyridine under the conditions of the King reaction gave pyridinium salts **25**, which upon basic hydrolysis and acidification provided acids **22**.[24] For purification acids **22** were converted to the corresponding methyl esters under standard conditions and the esters were purified by chromatography. Basic hydrolysis and acidification provided pure acids **22**.

JWH-414 (**9**, R = C_3H_7, R′ = H), JWH-415 (**9**, R = C_3H_7, R′ = CH_3), JWH-412 (**9**, R = C_5H_{11}, R′ = H), JWH-413 (**9**, R = C_5H_{11}, R′ = CH_3) were prepared from the acid chloride derived from commercial 4-fluoro-1-naphthoic acid by direct acylation of the appropriate indole. In the 3-(4-chloro-1-naphthoyl)indole series, the 1-propyl compound (**10**, JWH-400, R = C_3H_7, R′ = H) was prepared both by direct acylation of 1-propylindole and via alkylation of 3-(4-chloro-1-naphthoyl)indole. JWH-399 (**10**, R = C_3H_7, R′ = CH_3), JWH-398 (**10**, R = C_5H_{11}, R′ = H) and JWH-397 (**10**, R = C_5H_{11}, R′ = CH_3) were synthesized by alkylation of the requisite 3-acylindole. The four 3-(4-bromo-1-naphthoyl)indoles (**11**, JWH-386, JWH-395, JWH-387 and JWH-394) were prepared by both routes, while the 3-(4-iodo-1-naphthoyl)indoles (**12**, JWH-423, JWH-422, JWH-421 and JWH-420) were prepared by the acylation of the appropriate *N*-alkylindole with 4-iodo-1-naphthoyl chloride.

The substituted 1-naphthoic acids (**26**, **27** and **28**) needed for the synthesis of 3-(8-halo-1-napthoyl)indoles **14**–**16** were prepared from anhydro-8-(hydroxymercuri)-1-naphthoic acid (**29**) by a modification of the sequence reported by Whitmore many years ago and developed some years later by Shechter's group (Scheme 3).[25,26] The starting material, anhydro-8-(hydroxymercuri)-1-naphthoic acid, is prepared from 1,8-naphthoic anhydride (**30**) by treatment of the bis-sodium salt with mercuric acetate. For the preparation of 8-bromo- (**27**) and 8-iodo-1-naphthoic acid (**28**), treatment of anhydro acid **29** with an aqueous solution of sodium bromide (for the preparation of acid **15**) or potassium iodide (for the preparation of acid **16**) followed by addition of the appropriate halogen provided acids **27** and **28**.

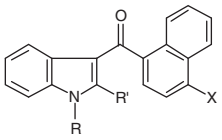
Although Whitmore reported that reaction of **29** with chlorine in glacial acetic acid gave 8-chloro-1-naphthoic acid (**26**),[25] Shechter found that this reaction afforded 5,8-dichloro-1-naphthoic acid as the major product[26] and in another early paper Rule and Barrett reported that chloromercuri compound **31** gave pure acid **26** in modest yield (31%) using Whitmore's procedure.[27] Acceptable yields of 8-chloro-1-naphthoic acid (**26**) were ultimately obtained using chlorine in acetic acid and carrying out the reaction at ice bath temperatures in acetic acid-dichloromethane.[28] In view of the rather lengthy synthesis of 8-fluoro-1-naphthoic acid, this compound was not prepared.[29]

The four 3-(8-chloro-1-naphthoyl)indoles (JWH-456, JWH-461, JWH-457, JWH-462), the 8-bromo analogs (JWH-428, JWH-429, JWH-424, JWH-425) and the 3-(8-iodo-1-naphthoyl)indoles (JWH-419, JWH-418, JWH-416 and JWH-417) were all prepared by the direct acylation of the appropriately substituted indole. In contrast to the 1-alkyl-3-(2-methoxy-1-naphthoyl)indole series, in which only those compounds derived form 2-methylindole (**13**) indicated restricted rotation about the naphthyl C-1 bond and the carbonyl carbon,[20] the ^1H NMR spectra of all of the 1-alkyl-3-(8-halo-1-naphthoyl)indoles (**14**–**16**) showed evidence of restricted rotation. Variable temperature ^1H NMR experiments from ambient temperature to 110 °C were carried out on several of these compounds and the data for 1-propyl-3-(8-bromo-1-naphthoyl)indole (JWH-428, **15**, R = C_3H_7, R′ = H) and its 2-methyl analog (JWH-429, **15**, R = C_3H_7, R′ = CH_3) are typical. At ambient temperature for **15** (R = C_3H_7, R′ = H) the methylene group adjacent to nitrogen appears as a multiplet at δ 4.14–4.18, while at 100 °C it is a well resolved triplet, J = 7.5 Hz, at δ 4.15. Also, in the aromatic region of the spectrum at ambient temperature, signals for two protons are absent. The differences in the spectra at ambient temperature and 100 °C for **15** (R = C_3H_7, R′ = CH_3) are considerably more pronounced with several protons giving rise to signals of reduced intensity at ambient temperature and the signal for the C-2 methyl group was absent. The signal for this methyl group was also absent at 75 °C, but appeared as a broadened singlet at 100 °C (see Section 5).[30]

The affinities of the 4- and 8-halonaphthoylindoles for the CB_1 and CB_2 receptors were determined by measuring their ability to displace the potent cannabinoid [^3H] CP-55,940 from its binding site in cloned human receptor preparations using the procedure described by Martin et al.[31] The results of these determinations are summarized in Tables 1 and 2. Also included in Table 1 are the receptor affinities for Δ^9-THC (**1**) and WIN-55,212-2 (**3**). The in vivo pharmacology was evaluated using the mouse model of cannabinoid activity, which measures spontaneous activity (SA), antinociception (as tail flick, TF), rectal temperature (RT) and catalepsy (as ring immobility).[32,33]

In the 4-halo-1-naphthoyl indole series (Table 1), CB_1 affinity was consistently and substantially better for each 1-pentyl-3-(1-naphthoyl)indole than for the corresponding 1-propyl compound. For example, the K_i value for JWH-412, 1-pentyl-3-(4-fluoro-1-

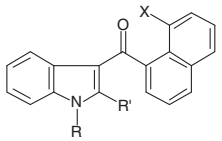
Table 1
In vitro and in vivo pharmacology of 1-alkyl-3-(4-halo-1-naphthoyl)indoles (**9**–**12**), Δ^9-THC (**1**), JWH-015 (**5**), JWH-018 (**4**) and WIN-55,212-2 (**3**)



Compound	R	R′	X	K_i (nM)			ED_{50} (μmol/kg)			
				CB_1	CB_2	Ratio	Tetrad tests in mice[f]			
						CB_1/CB_2	SA	TF	RT	RI
Δ^9-THC (**1**)				41 ± 2[a]	36 ± 10[b]	1.1	3.2	4.5	4.5	4.8
WIN-55,212-2 (**3**)				1.9 ± 0.1[b]	0.28 ± 0.16[b]	6.8	0.23[c]	0.94[c]	28[c]	2.8[c]
JWH-015 (**5**)				164 ± 22[d]	13,8 ± 4.6[d]	11.9	18.7[e]	84.7[e]	99.1[e]	87.2[e]
JWH-018 (**4**)				9 ± 5[d]	2.9 ± 2.6[d]	3.2	0.44[e]	0.09[e]	1.7[e]	3–9[e]
JWH-414	C_3H_7	H	F	240 ± 7	33 ± 2	7	67% at 91	59% at 91	-3.3 at 91	Not active
JWH-415	C_3H_7	CH_3	F	530 ± 37	38 ± 1	14	9.6	11.9	30.1	23.2
Rimonabant (3 mg/kg) blocked or partially blocked all in vivo effects of 30 mg/kg of JWH-415										
JWH-412	C_5H_{11}	H	F	7.2 ± 0.5	3.2 ± 0.5	2	<0.08	0.14	<0.08	0.58
JWH-413	C_5H_{11}	CH_3	F	14 ± 0.7	2.2 ± 0.2	6	0.38	0.48	0.54	0.99
JWH-400	C_3H_7	H	Cl	93 ± 8	44 ± 0.4	2	Not tested			
JWH-399	C_3H_7	CH_3	Cl	187 ± 16	22 ± 1	9	70% at 83	100% at 83	−5.4 at 83	70% at 83
JWH-398	C_5H_{11}	H	Cl	2.3 ± 0.1	2.8 ± 0.2	0.8	82% at 27	90% at 27	−4.9 at 27	67% at 27
JWH-397	C_5H_{11}	CH_3	Cl	8.9 ± 0.3	2.3 ± 0.02	4	94% at 77	100% at 77	−6.4 at 77	88% at 77
JWH-386	C_3H_7	H	Br	161 ± 16	27 ± 1.6	6	71% at 77	75% at 77	−3.9 at 77	26% at 77
JWH-395	C_3H_7	CH_3	Br	373 ± 43	31 ± 2	12	80% at 74	96% at 74	−5.0 at 74	50% at 74
JWH-387	C_5H_{11}	H	Br	1.2 ± 0.1	1.1 ± 0.1	1	Not tested			
JWH-394	C_5H_{11}	CH_3	Br	14 ± 0.2	2.8 ± 0.2	5	Not tested			
JWH-423	C_3H_7	H	I	140 ± 10	6.6 ± 0.2	21	10% at 6.8	26% at 6.8	−1.7 at 6.8	2% at 6.8
JWH-422	C_3H_7	CH_3	I	501 ± 48	20 ± 0.4	25	74% at 66	60% at 66	−3.0 at 66	40% at 66
JWH-421	C_5H_{11}	H	I	2.5 ± 0.2	1.3 ± 0.02	2	0.30	0.45	0.19	0.38
JWH-420	C_5H_{11}	CH_3	I	14 ± 1	2.1 ± 0.1	7	1.1	2.5	2.6	2.5

[a] Ref.[41]
[b] Ref.[15]
[c] Ref.14
[d] Ref.[20]
[e] Ref.[18]
[f] Pharmacological tests in mice included spontaneous activity (SA), tail flick test of antinociception (TF), change in rectal temperature (RT), and ring immobility (RI).

Table 2
Receptor affinities (mean ± SEM) of 1-alkyl-3-(8-halo-1-naphthoyl)indoles (**14**–**16**)



Compound	R	R′	X	K_i (nM)			ED_{50} (μmol kg)			
				CB_1	CB_2	Ratio CB_1/CB_2	SA	TF	RT	RI
JWH-456	C_3H_7	H	Cl	642 ± 50	134 ± 20	4.8	68% at 86	72% at 86	Not active at 86	
JWH-461	C_3H_7	CH_3	Cl	>10,000	174 ± 29	>57	70% at 83	42% at 83	−2.3 at 83	15% at 83
JWH-457	C_5H_{11}	H	Cl	23 ± 1	10 ± 0.8	2.3	94% at 80	100% at 80	−6.9 at 80	92% at 80
JWH-462	C_5H_{11}	CH_3	Cl	116 ± 7	19 ± 1.6	6.1	84% at 77	100% at 77	−5.5 at 77	86% at 77
JWH-428	C_3H_7	H	Br	>10,000	192 ± 14	>52	76% at 77	9% at 77	−2 at 77	5% at 77
JWH-429	C_3H_7	CH_3	Br	>10,000	278 ± 69	>36	49% at 74	72% at 74	−2.3 at 74	4% at 74
JWH-424	C_5H_{11}	H	Br	21 ± 3.4	5.4 ± 0.2	4	1.4	1.8	2.4	3.7
JWH-425	C_5H_{11}	CH_3	Br	54 ± 11	10 ± 0.4	5.5	79% at 6.9	100% at 6.9	−3.7 at 6.9	53% at 6.9
JWH-419	C_3H_7	H	I	2960 ± 240	152 ± 33	19	−25% at 68	Not active at 68		
JWH-418	C_3H_7	CH_3	I	4290 ± 440	536 ± 66	8	Not active at 66			
JWH-416	C_5H_{11}	H	I	73 ± 10	3.3 ± 0.1	22	1.8	1.3	~6.4	3.6
JWH-417	C_5H_{11}	CH_3	I	522 ± 58	13 ± 0.2	40	Not active at 6.2			

naphthoyl)indole, was 7.2 nM compared to a K_i of 240 nM for JWH-414, 1-propyl-3-(4-fluoro-1-naphthoyl)indole. CB_1 affinities of the 1-propyl-3-(4-halo-1-naphthoyl)indoles were moderate, ranging from 93 to 240 nM, while the 1-pentyl-3-(4-halo-1-naphthoyl)indoles had good affinities, ranging from 1.2 to 7.2 nM. A similar pattern of differential affinities as a function of the length of the alkyl substituent was observed for the 1-alkyl-2-methyl-3-(4-halo-1-naphthoyl)indole series; however, the presence of a 2-methyl substituent substantially decreased CB_1 affinities for compounds with either alkyl length. While the resulting CB_1 affinities for the 1-propyl analogs were moderate to poor, with K_is ranging from 187 to 530 nM, the 1-pentyl compounds retained good CB_1 affinity, with K_i values ranging from 8.9 to 14 nM. Electronegativity of the 4-halogen substituent also affected CB_1 affinity. 4-Chloro substitution produced the best CB_1 affinity in each group, K_i = 93 nM for JWH-400, 1-propyl-3-(4-chloro-1-naphthoyl)indole; K_i = 187 nM

for JWH-399, 1-propyl-2-methyl-3-(4-chloro-1-naphthoyl)indole; K_i = 8.9 nM for JWH-397, 1-pentyl-2-methyl-3-(4-chloro-1-naphthoyl)indole, except for the 1-pentyl-3-(4-halo-1-naphthoyl)-indoles, in which 3-(4-bromo-1-naphthoyl) substitution resulted in optimal CB_1 affinity (K_i = 1.2 for JWH-387). Increasing electronegativity through substitution of a 3-(4-fluoro-1-naphthoyl) substituent greatly lessened CB_1 affinity, K_i = 240 nM for JWH-414, 1-propyl-3-(4-fluoro-1-naphthoyl)indole; K_i = 7.2 nM for JWH-412, 1-pentyl-3-(4-fluoro-1-naphthoyl)indole; K_i = 530 nM for JWH-415, 1-propyl-2-methyl-3-(4-fluoro-1-naphthoyl)indole and K_i = 14 nM for JWH-413, 1-pentyl-2-methyl-3-(4-fluoro-1-naphthoyl)indole. Substitution of a less electronegative iodo substituent in 1-propyl-2-methyl-3-(4-iodo-1-naphthoyl)indole, JWH-422 (K_i = 501 nM) has a similar effect. For the other 1-propyl-3-(4-halo-1-naphthoyl)-indoles, 1-pentyl-3-(4-halo-1-naphthoyl)indoles, and 1-pentyl-2-methyl-3-(4-halo-1-naphthoyl)indoles, the CB_1 receptor affinities for 3-(4-chloro, bromo,iodo-1-naphthoyl) indoles do not vary widely within each series.

Regardless of their CB_1 affinities, all of the 3-(4-halo-1-naphthoyl)indoles have good CB_2 affinities. Further, whereas THC shows similar affinities for both CB_1 and CB_2 receptors, WIN-55,212-2 and the majority of the 3-(4-halo-1-naphthoyl)indoles [except for two, JWH-398 and JWH-387, 1-pentyl-3-(4-chloro-1-naphthoyl)indole and 1-pentyl-3-(4-bromo-1-naphthoyl)indole, respectively] are mildly to moderately CB_2 selective, with CB_1/CB_2 ratios ranging from 2 to 25. The 1-propyl analogs exhibit better CB_2 selectivity than corresponding 1-pentyl compounds regardless of whether they have a methyl substitution at the indole 2-position or are unsubstituted. However, compared to the analogs that are unsubstituted at the 2-position of the indole group, CB_2 selectivity (but not absolute affinity) is increased with addition of the 2-methyl substituent for either the 1-propyl or 1-pentyl indoles. The best absolute values for CB_2 affinities are exhibited by 1-pentyl-3-(4-halo-1-naphthoyl)indoles, a pattern that was also observed for CB_1 affinities. For the 1-pentyl-3-(4-halo-1-naphthoyl)indole series, CB_2 affinities ranged from 1.1 to 13 nM, with the best affinity obtained for JWH-387, 1-pentyl-3-(4-bromo-1-naphthoyl)indole. Decreasing electronegativity by substitution of a 4-iodo-1-naphthoyl group correspondingly decreases CB_2 affinity (JWH-421, K_i = 13 nM). Although increasing electronegativity by 4-chloro-1-naphthoyl or 4-fluoro-1-naphthoyl substitution also decreases CB_2 affinity, the magnitude of these decreases is much less [K_i = 2.8 nM for JWH-398, 1-pentyl-3-(4-chloro-1-naphthoyl)indole and K_i = 3.2 nM for JWH-412, 1-pentyl-2-*H*-3-(4-fluoro-1-naphthoyl)indole]. CB_2 affinities for the 1-pentyl-2-methyl-3-(4-halo-1-naphthoyl)indole series vary little across 4-halogen substitutions, ranging from 2.1 to 2.8 nM. In contrast, CB_2 affinities for the 1-propyl-3-(4-halo-1-naphthoyl)indoles range from 6.6 nM for 1-propyl-3-(4-iodo-1-naphthoyl)indole, JWH-423, to 44 nM for 1-propyl-2-*H*-3-(4-chloro-1-naphthoyl)indole, JWH-400. The remaining 1-propyl indoles possess CB_2 affinities that are similar (20–33 nM).

Because limited yields prevented determination of full dose-effect curves, assessment of in vivo potency was not possible for many of the 4-halo-1-naphthoyl indoles; however, tests with probe doses (3–30 mg/kg) suggested that compounds with good to moderate CB_1 affinity exhibit cannabinoid activity in the in vivo test battery. Where potency calculations were possible, compounds with good CB_1 affinities also possess good potencies in each of the four in vivo tests, with one exception. JWH-415, 1-propyl-2-methyl-3-(4-fluoro-1-naphthoyl)indole, has poor CB_1 affinity (K_i = 530 nM); yet, it produces all characteristic cannabinoid effects, albeit potencies are lower compared to other compounds with better CB_1 affinities. Further, 3 mg/kg rimonabant blocked or partially blocked all tetrad effects of 30 mg/kg JWH-415, suggesting that these effects were CB_1 receptor-mediated. To evaluate possible receptor mediation of the in vivo effects of JWH-415, the compound was assessed in a [^{35}S]GTPγS assay to determine its ability to activate the CB_1 receptor. JWH-415 produces G-protein activation in a concentration-dependent manner, with an EC_{50} of 268 μM (±42 SEM). Efficacy, however, is only partial (E_{max} = 59% ± 3).

Table 2 presents a summary of the pharmacological results for the 1-alkyl-3-(8-halo-1-naphthoyl)indoles. All of these compounds possess substantially less affinity for both CB_1 and CB_2 receptors than did the comparable 1-alkyl-3-(4-halo-1-naphthoyl)indoles, albeit some of the SAR patterns that were observed in the 4-halogen series are also observed in the 8-halogen series. First, the 1-pentyl-3-(8-halo-1-naphthoyl)indoles exhibit better CB_1 and CB_2 affinities than the 1-propyl-3-(8-halo-1-naphthoyl)indoles. The best CB_1 affinity for the 1-propyl series is a K_i of 642 nM for JWH-456, 1-propyl-3-(8-chloro-1-naphthoyl)indole. Several of the compounds fail to demonstrate measurable binding to the CB_1 receptor [K_i >10,000 for JWH-428, 1-propyl-3-(8-bromo-1-naphthoyl)indole, JWH-461, 1-propyl-2-methyl-3-(8-chloro-1-naphthoyl)indole, and JWH-429, 1-propyl-2-methyl-3-(8-bromo-1-naphthoyl)indole]. In contrast, CB_1 affinities for the 1-pentyl-3-(8-halo-1-naphthoyl)indoles range from 21 to 73 nM and 54 to 522 nM for the 1-pentyl-2-methyl-3-(8-halo-1-naphthoyl)indole series. CB_2 affinities are also better for the 1-pentyl-3-(8-halo-1-naphthoyl)indoles than for the 1-propyl-3-(8-halo-1-naphthoyl)indoles, ranging from K_i = 3.3–10 nM for the 1-pentyl-3-(8-halo-1-naphthoyl)indoles and K_i = 10–19 nM for the 1-pentyl-2-methyl-3-(8-halo-1-naphthoyl)indoles. For the propyl analogs, CB_2 affinities range from 102 to 152 nM for the 1-propyl-3-(8-halo-1-naphthoyl)indoles and from 174 to 536 nM for the 1-propyl-2-methyl-3-(8-halo-1-naphthoyl)indoles. As was the case for the compounds with 4-halo-1-naphthoyl substitutions, all compounds are CB_2 selective. In addition, methylation at the 2-position of the indole group results in decreased CB_1 affinities, as it did for the 4-halogen series. In the 1-pentyl-3-(8-halo-1-naphthoyl) series, a moderate degree of electronegativity appears to optimize CB_1 affinity, with the caveat that synthesis of the highly negative 1-alkyl-3-(8-fluoro-1-naphthoyl)indoles was impractical. Hence, JWH-457, 1-pentyl-3-(8-chloro-1-naphthoyl)indole, JWH-424, 1-pentyl-3-(8-bromo-1-naphthoyl)indole and JWH-425, 1-pentyl-2-methyl-3-(8-bromo-1-naphthoyl)indole, have the best CB_1 affinities, with K_i = 23, 21 and 54 nM, respectively. JWH-425 also has the best CB_2 affinity of the 1-pentyl-2-methyl-3-(8-halo-1-naphthoyl)indoles, with K_i = 10 nM, whereas the best CB_2 affinity of the 1-pentyl-3-(8-halo-1-naphthoyl)indoles is JWH-416, 1-pentyl-3-(8-iodo-1-naphthoyl)-indole, with K_i = 3.3 nM. The only compound that produced the full cannabinoid profile in vivo was JWH-424, 1-pentyl-3-(8-bromo-1-naphthoyl)indole). JWH-457, 1-pentyl-3-(8-chloro-1-naphthoyl)indole, which also has good CB_1 receptor affinity (K_i = 23 nM) is much less potent in vivo than would be expected on the basis of its CB_1 affinity.

3. Discussion

The primary purpose of this study was to examine steric and electronic effects on CB_1 and CB_2 receptor affinities and in vivo pharmacology of moderately electron withdrawing halogen substituents at the naphthoyl C-4 or C-8 positions in a series of 1-alkyl-3-(1-naphthoyl)indoles. In addition, two electroneutral structural features (the length of the *N*-alkyl group and the presence of a methyl at the 2-indole position) were manipulated and were found to strongly affect affinities at both cannabinoid receptors for this series of compounds. 1-Pentyl-3-(1-naphthoyl)indoles consistently exhibit more favorable CB_1 affinity than 1-propyl-3-(1-naphthoyl)indoles with the corresponding C-4 or C-8 substitutions. Whereas CB_1 affinities range from good for 1-pentyl-3-(1-naphthoyl)indoles with C-4 substitutions (1.2–14 nM) to moderate

or poor for those with C-8 substitutions (21–522 nM), CB_1 affinities for 1-propyl-3-(1-naphthoyl)indoles are moderate or poor (93–530 nM) for C-4 substitutions and are consistently poor or negligible for propyl compounds with C-8 substitutions. Absolute values obtained for CB_2 affinity are also better for 1-pentyl-3-(1-naphthoyl)indoles both those with C-4 and C-8 substituents, ranging from 1.1 nM for 1-pentyl-3-(4-bromo-1-naphthoyl)indole (JWH-387) to 19 nM for 1-pentyl-2-methyl-3-(8-chloro-1-naphthoyl)indole (JWH-462). However, the combination of the decrease in CB_1 affinities provided by a 1-propyl group combined with relatively little decrease in CB_2 affinity leads to greater CB_2 selectivity for the 1-propyl-3-(1-naphthoyl)indoles than for those indoles with an *N*-pentyl group. Greater CB_2 selectivity appears to be a function of the length of the *N*-alkyl group, as similar selectivity for CB_2 receptors was found for JWH-015 (**5**), a 1-propyl-3-(1-naphthoyl)indole derivative that is unsubstituted on the naphthoyl moiety.[15,19] In addition to the length of the alkyl group, the presence of a methyl at the indole 2-position also affects CB_1 affinity. In the compounds with C-4 substitutions, the presence of a 2-methyl substituent decreases CB_1 affinity without having substantive effect on CB_2 affinity; hence, the net result of this substitution is increased CB_2 selectivity. In contrast, 2-methyl substitution in the series of compounds with C-8 substitutions decreases CB_2 affinity in the set of 1-propyl analogs, and to a lesser extent, in the set of 1-pentyl analogs, with the most pronounced decrease seen for 1-propyl-2-methyl-3-(8-iodo-1-naphthoyl)indole (JWH-418). In summary, the results observed with the two electroneutral structural manipulations are consistent with a number of previous SAR investigations of indole- and pyrrole-derived cannabinoids in which these structural manipulations produce similar patterns of alterations of CB_1 and CB_2 affinities and selectivity and suggest that length of the alkyl substituent is as crucial for this cannabinoid structural motif as it is for cannabinoids based on the tetrahydrocannabinol structural motif.[2,19,21]

The in vivo evaluation of the series of naphthoylindoles with C-4 and C-8, that was undertaken for those compounds for which sufficient sample was available, revealed that efficacy for producing cannabimimetic effects in mice is associated with good CB_1 affinity, as has been shown previously for structural analogs based on a tetrahydrocannabinol template as well as for other indole and pyrrole cannabinoid series.[18,34] One anomaly in the current series was JWH-415, 1-propyl-2-methyl-3-(4-fluoro-naphthoyl)indole. Although the CB_1 affinity of JWH-415 is poor (K_i = 530 nM), it exhibited rimonabant-reversible activity in the battery of mouse tests. Further investigation showed that JWH-415 is a low efficacy agonist at the CB_1 receptor. A similar finding was reported previously for several 1-deoxy-THC analogs, with the most prominent being deoxy-$\Delta^{9(11)}$-THC-dimethylheptyl (JWH-104). Similar to the results observed here with JWH-415, JWH-104 has low CB_1 affinity (K_i = 909 nM); yet, it produced cannabimimetic effects in the battery of mouse tests that were blocked by rimonabant.[35] Results of a [^{35}S]GTPγS binding assay showed that JWH-104 also functions as a low efficacy agonist at CB_1 receptors. Interestingly, JWH-104 did not substitute for THC in a drug discrimination procedure in mice (an animal model of marijuana-like intoxication),[36,37] suggesting that it does not produce the entire complement of cannabinoid effects seen with THC. JWH-415 has not been tested in the latter behavioral test.

To date, the only substituents on the naphthoyl moiety that had been examined in indole-derived cannabinoids were weakly to moderately electron releasing. In the present study, moderately electron withdrawing C-4 napthoyl substituents with a range of electronegativity (F, Cl, Br, and I) were synthesized and evaluated. Results show that moderate electronegativity is favorable for CB_1 affinity, with chloro analogs having the best, but modest, affinity in the series of 1-propyl analogs (JWH-400 and JWH-399) and 1-pentyl-2-methyl analogs (JWH-397). The 4-bromonaphthoyl analog (JWH-387) has the best affinity of the 1-pentyl analogs that are unsubstituted at C-2 of the indole. Fluorine is the most electronegative of the halogens and a C-4 fluoro substituent suppresses CB_1 affinity in the 1-propyl analogs, with or without a 2-methyl substituent on the indole core (JWH-415 and JWH-414, respectively). The least electronegative halogen is iodine and an iodo substituent at C-4 also reduces CB_1 affinity somewhat for the 1-propyl-2-methyl analog, JWH-422. However, the other 4-iodonaphthoyl compounds have CB_1 receptor affinities that are similar to those of the chloro and bromo analogs. Previous work has shown that 4-fluorobenzyl moiety in place of the naphthoyl substituent in a series of 1-pentyl-phenylacetylindoles[23] or for the hydroxy group of THC in a series of fluorinated classical cannabinoid analogs[38] substantially decreases CB_1 receptor affinity and in vivo potency. CB_2 affinity is less affected by electronegativity, particularly in the 1-pentyl and 1-pentyl-2-methyl series where CB_2 affinities are uniformly good and range from 1.1 to 3.2 nM.

In the series of 3-(8-halo-1-naphthoyl)indoles, iodine, the least electronegative halogen, decreases CB_1 affinity in the 1-pentyl analogs, both those with and without a methyl group at the 2-position of the indole (JWH-417 and JWH-416, respectively). The CB_1 receptor affinities for the 1-propyl analogs with halogen substituents C-8 are from negligible to very low across the entire range of negativities evaluated (i.e., Cl, Br, and I).

In addition to electronic effects, it is likely that steric effects of the C-8 substitutions contribute to the low CB_1 affinities and CB_2 selectivity of this series of compounds. In earlier work it was found that 1-propyl- and 1-pentyl-2-methyl-3-(2-methoxy-1-naphthoyl)-indoles (**13**) have restricted rotation about the naphthalene C-1 bond to the carbonyl carbon.[20] In addition, two of the compounds in this series were very selective for the CB_2 receptor. While molecular modeling studies of the 3-(8-halogenated-1-naphthoyl)indole derivatives were not carried out, their consistently lower CB_1 affinity (compared to compounds with corresponding C-4 substitutions) suggests that steric hinderance of rotation caused by the C-8 substitution interferes with interaction with the CB_1 receptor, particularly for those compounds with an indole C-2 methyl substituent. The ^{1}H NMR spectra of these compounds is in agreement with this hypothesis, see above and the experimental section.[30] This is particularly apparent with a relatively large iodo substituent at C-8 of the naphthoyl moiety. A possible mechanism for the decrease in CB_1 affinity is that substitution at C-8 of the naphthoyl ring prevents optimal aromatic stacking of these compounds with the aromatic amino acids of the CB_1 receptor. These interactions have been shown to be important for the binding of cannabimimetic indoles to the CB_1 receptor.[20,39] Similar positional biases also occurred in series of 1-pentyl-3-phenylacetylindoles and 1-alkyl-2-aryl-4-(1-naphthoyl)pyrroles with electron withdrawing substituents.[23,40] With all substituents CB_1 and CB_2 affinities were best with substitution at the 2-position of the phenyl or pyrrole moieties, with intermediate affinities for the 3-position and with the least affinity for compounds with 4-substituents, suggesting that positional substitutions following conversion of the indole template to a pyrrole produce similar changes in affinities as deletion of one of the naphthoyl rings (i.e., conversion to a phenylacetyl) with the same positional substitutions on the opposite side of the molecule. Hence, the position of substituents on either side of the molecule appears to be important for CB_1 receptor affinity and in vivo potency for indole-derived cannabinoids, as it has been shown to be for synthetic cannabinoids based upon a THC structural template.[41] For example, compounds with halogen (e.g., iodo, bromo, fluoro) or nitrogen substitutions at the C-2 and 4-aryl positions (on either side of the C-3 pentyl side chain of THC) had lower CB_1 receptor affinity and were less potent in vivo than compounds with similar substitutions at the terminal end of the side chain.[42] In addition, the number of aromatic substituents also may play a role in interaction with the CB_1 receptor, as suggested by the finding that the series of 1-pentyl-3-phenylacetylindoles shows

attenuated CB_1 receptor affinity compared to their 1-pentyl-3-naphthoylindole congeners.[20]

4. Conclusions

The major structural manipulations in the present series included halogen substitution on the 3-naphthoyl group (i.e., fluoro, chloro, bromo, and iodo), the position of the substituent on the naphthoyl (i.e., C-4 or C-8), length of the *N*-alkyl group (i.e., propyl or pentyl), and presence of a methyl group at the 2-position of the indole nucleus. Of these factors, the position of the halogen substituent on the naphthoyl group and the length of the *N*-alkyl group were the most critical factors affecting CB_1 and CB_2 affinities, with C-4 and 1-pentyl substitutions, respectively, producing consistently good affinities at both receptors. While CB_2 selectivity was enhanced by 1-propyl (vs 1-pentyl) substitution, this effect occurred primarily because of a corresponding decrease in CB_1 affinity rather than an improvement in CB_2 affinity. Together, these results suggest that steric effects play a far more prominent role in determining the nature of the interaction of this series of 1-alkyl-3-(1-naphthoyl)indoles with the identified cannabinoid receptors than do electronic effects.

5. Experimental

5.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300AC spectrometer or a 500 MHz Bruker Fourier Transform Spectrometer. Mass spectral analyses were performed on a Hewlett–Packard 5890A capillary gas chromatograph equipped with a mass sensitive detector or a Shimadzu QP2010 capillary gas chromatograph/mass spectrometer. 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 Sorbent Technologies silica gel (32–63 μ) using the indicated solvents as eluents. All new compounds were homogeneous to TLC and ^{13}C NMR. All target compounds were homogeneous to GLC or TLC in two different solvent systems. TLC was carried out using 200 μm silica gel plates using the indicated solvents. GLC analyses were performed on the Hewlett-Packard 5890A GC/MS or the Shimadzu QP2010 using a 60 m carbowax column and helium gas as a carrier. An initial column temperature of 60 °C was employed and the temperature was increased at a rate of 1.5 °C/min to a maximum temperature of 300 °C with a total run time of 20 min.

5.1.1. 1-Propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414, 9, R = C_3H_7, R′ = H, X = F)

To a solution of 0.07 g (0.44 mmol) of 1-propylindole in 5 mL of dry dichloromethane under Ar was added 0.60 mL (0.60 mmol), of 1 M dimethylaluminum chloride in hexanes and the mixture was stirred for 30 min at 0 °C. To this mixture was added dropwise a solution of freshly prepared 4-fluoro-1-naphthoyl chloride in 3 mL of dry dichloromethane. The acid chloride was prepared by the addition of 3 mL (41 mmol) of thionyl chloride to 0.12 g (0.63 mmol) of 4-fluoro-1-naphthoic acid under Ar. The mixture was heated at reflux for 2 h, cooled to ambient temperature and the thionyl chloride was removed in vacuo. The acid chloride was added to the indole mixture without further purification and the mixture was stirred at ambient temperature overnight. The reaction was quenched with water and extracted with ether. The ethereal solution was washed with water and 1 M KOH, dried ($MgSO_4$) and the solvent was removed in vacuo to give the crude product, which was purified by column chromatography (petroleum ether/ether, 8:2) to give 0.076 g (52%) of 1-propyl-3-(4-fluoro-1-naphthoyl)indole as a tan solid: mp 166–167 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.94 (t, J = 7.3 Hz, 3H), 1.87 (sextet, J = 7.3 Hz, 2H), 4.09 (t, J = 7 Hz, 2H), 7.21 (t, J = 8.8 Hz, 1H), 7.40–7.43 (m, 4H), 7.57 (t, J = 7 Hz, 1H), 7.62 (t, J = 7 Hz, 1H), 7.66 (t, J = 6.5 Hz, 1H), 8.22 (d, J = 8 Hz, 1H), 8.29 (d, J = 8 Hz, 1H), 8.51–8.53 (m, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 11.3, 23.1, 48.8, 108.1, 108.3, 110.1, 117.5, 120.7, 120.7, 122.9, 122.9, 123.7, 126.0, 126.4, 126.4, 126.7, 127.0, 127.8, 137.1, 137.8, 191.1; GC/MS (EI) *m/z* (rel intensity) 331 (100), 302 (66), 274 (12), 186 (95), 144 (47); HRMS (ES^+) *m/z* calcd for $C_{22}H_{18}FNO$: 331.1372; found: 331.1367.

5.1.2. 2-Methyl-1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-415, 9, R = C_3H_7, R′ = CH_3, X = F)

JWH-415 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.08 g (0.45 mmol) of 2-methyl-1-propylindole and 0.13 g (0.68 mmol) of 4-fluoro-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ether, 8:2) 0.089 g (56%) of 2-methyl-*N*-propyl-3-(4-fluoro-1-naphthoyl)indole as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, J = 7.5 Hz, 3H), 1.82 (sextet, J = 7.5 Hz, 2H), 2.51 (s, 3H), 4.09 (t, J = 7.5 Hz, 2H), 6.99 (t, J = 7.5 Hz, 1H), 7.16 (q, J = 8.3 Hz, 3H), 7.31 (d, J = 8.3 Hz, 1H), 7.50 (t, J = 7.4 Hz, 1H), 7.53–7.56 (m, 2H), 8.17–8.20 (m, 2H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 11.5, 12.6, 23.0, 44.9, 108.7, 108.8, 109.6, 114.9, 120.7, 120.8, 121.1, 121.9, 122.3, 125.7, 126.7, 126.7, 127.1, 127.9, 136.1, 136.5, 145.6, 190.5; GC/MS (EI) *m/z* (rel intensity) 345 (100), 328 (34), 288 (47), 173 (73); HRMS (ES^+) *m/z* calcd for $C_{23}H_{20}FNO$: 345.1529; found: 345.1531.

5.1.3. 1-Pentyl-3-(4-fluoro-1-naphthoyl)indole (JWH-412, 9, R = C_5H_{11}, R′ = H, X = F)

JWH-412 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.070 g (0.38 mmol) of 1-pentylindole and 0.11 g (0.58 mmol) of 4-fluoro-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 7:3) 0.021 g (10%) of 1-pentyl-3-(4-fluoro-1-naphthoyl)indole as a brown oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, J = 7 Hz, 3H), 1.28–1.35 (m, 4H), 1.85 (p, J = 7 Hz, 2H), 4.11 (t, J = 7 Hz, 2H), 7.22 (dd, J = 8, 10 Hz, 1H), 7.38–7.40 (m, 3H), 7.42–7.43 (m, 1H), 7.56 (t, J = 7 Hz, 1H), 7.61 (t, J = 7 Hz, 1H), 7.66 (dd, J = 5.5, 8 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 8.28 (d, J = 8.5 Hz, 1H), 8.48–8.50 (m, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.9, 22.2, 28.9, 29.5, 47.2, 108.1, 108.3, 110.0, 117.5, 120.6, 120.7, 122.9, 122.9, 123.7, 126.0, 126.4, 126.7, 127.0, 127.8, 132.6, 137.1, 137.7, 191.0; GC/MS (EI) *m/z* (rel intensity) 359 (50), 343 (22), 302 (54), 281 (26), 207 (100); HRMS (ES^+) *m/z* calcd for $C_{24}H_{22}FNO$: 359.1685; found: 359.1679.

5.1.4. 2-Methyl-1-pentyl-3-(4-fluoro-1-naphthoyl)indole (JWH-413, 9, R = C_5H_{11}, R′ = CH_3, X = F)

JWH-413 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.10 g (0.48 mmol) of 2-methyl-1-pentylindole and 0.14 g (0.74 mmol) of 4-fluoro-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ether, 9:1) 0.022 g (12%) of 2-methyl-1-pentyl-3-(4-fluoro-1-naphthoyl)indole as a green oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, J = 7 Hz, 3H), 1.41–1.42 (m, 4H), 1.81–1.85 (m, 2H), 2.56 (s, 3H), 4.16 (t, J = 7.5 Hz, 2H), 7.03 (d, J = 8 Hz, 1H), 7.15–7.22 (m, 4H), 7.34 (d,

J = 8.5 Hz, 1H), 7.54–7.60 (m, 3H), 8.22 (d, J = 9 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 12.6, 13.9, 22.4, 29.1, 29.4, 43.4, 108.6, 108.8, 109.5, 114.9, 120.7, 120.7, 121.1, 121.9, 122.2, 123.9, 124.1, 125.7, 126.6, 126.7, 127.1, 127.9, 136.1, 145.5, 192.4; GC/MS (EI) m/z (rel intensity) 373 (74), 358 (45), 316 (44), 173 (100), 145 (55); HRMS (ES^+) m/z calcd for $C_{25}H_{24}FNO$: 373.1842; found: 373.1850.

5.1.5. 1-Acetyl-4-chloro-naphthalene (24, X = Cl)

To a solution of 0.15 g (1.9 mmol) of acetyl chloride in 8 mL of freshly distilled dichloromethane under N_2 was added 0.26 g (2.0 mmol) of $AlCl_3$. This mixture was stirred at ambient temperature for 5 min and 0.28 g (1.72 mmol) of 1-chloronaphthalene was added dropwise over 10 min. The mixture was heated to 40 °C and stirred for 1 h, poured over a mixture of ice and concentrated HCl. The product was extracted into ether, washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo to give 0.56 g (84%) of 1-acetyl-4-chloro-naphthalene as a yellow oil, which was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.58 (s, 3H), 7.33 (d, J = 7.8 Hz, 1H), 7.48–7.55 (m, 2H), 7.57 (d, J = 7.8 Hz, 1H), 8.16 (d, J = 7.8, 1H), 8.72 (d, J = 7.2, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 29.8, 124.5, 124.7, 126.4, 127.4, 128.4, 128.6, 130.9, 131.2, 134.1, 136.6, 200.5; GC/MS (EI) m/z (rel intensity) 204 (69), 189 (100), 161 (75), 126 (54). The data agree in all respects with those reported previously.[43]

5.1.6. 4-Chloro-1-naphthoic acid (22, X = Cl)

To a solution of 2.44 g (11.9 mmol) of 1-acetyl-4-chloronaphthalene in 5 mL of freshly distilled pyridine under N_2 was added 3.33 g (13.1 mmol) of I_2 dissolved in 15 mL of freshly distilled pyridine. The mixture was heated at reflux for 40 min, cooled to ambient temperature and diluted with ether until a brown precipitate formed. The precipitate was filtered off, suspended in 12 mL of 6 M aqueous NaOH and heated at reflux for 2 h. After cooling the solution was acidified with 10% HCl, the crude 4-chloro-1-naphthoic acid was extracted into ether and the ethereal extract was washed with brine. After drying ($MgSO_4$) the solution was concentrated in vacuo. The product was dissolved in 25 mL of MeOH to which 5 mL of concentrated H_2SO_4 was cautiously added. The solution was heated at reflux for 3 h. After cooling to ambient temperature the crude ester was extracted into ether, the ethereal solution was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo to give 1.66 g (63%) of methyl 4-chloro-1-naphthoate as a brown oil, which was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90 (s, 3H), 7.38 (d, J = 7.8, 1H), 7.45–7.56 (m, 2H), 7.87 (d, J = 8.1, 1H), 8.18 (d, J = 8.4, 1H), 8.93 (d, J = 8.1, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 52.1, 124.6, 124.9, 125.8. 126.2, 127.1, 128.3, 129.9, 130.7, 132.3, 137.2, 166.9; GC/MS (EI) m/z (rel intensity) 220 (61), 189 (100), 161 (48), 126 (42).

A mixture of 0.25 g (1.1 mmol) of methyl 4-chloro-1-naphthoate and 2.10 g (37.4 mmol) of KOH in 20 mL of H_2O was heated at reflux for 12 h under N_2. The reaction mixture was cooled to ambient temperature, acidified with concentrated HCl and the product was extracted into ether and dried ($MgSO_4$). The organic phase was concentrated in vacuo to give 0.27 g (88%) of 4-chloro-1-naphthoic acid as an off-white solid: mp 223–224 °C (lit mp 220–221 °C[44]); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74–7.81 (m, 3H), 8.11 (d, J = 6 Hz, 1H), 8.28–8.31 (m, 1H), 8.93–8.97 (m, 1H), 13.8 (br s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 124.7, 126.1, 126.7, 127.9, 128.3, 129.0, 130.4, 130.6, 132.3, 135.7, 168.5.

5.1.7. 3-(4-Chloro-1-naphthoyl)indole (18, R = H, Ar = 4-chloronaphthyl)

A solution of 0.48 mL (1.45 mmol) of EtMgBr [3.0 M in diethyl ether]) was added with stirring to 3 mL of dry ether at 0 °C under Ar and stirred until it became cloudy white. A solution of 0.12 g (1.06 mmol) of indole in 2 mL of dry ether was added and the mixture was stirred at 0 °C for 30 min followed by stirring at ambient temperature for 30 min. A solution of freshly prepared 4-chloro-1-naphthoyl chloride in 3 mL of dry ether was added dropwise via syringe. The acid chloride was prepared by the addition of 3 mL (41 mmol) of thionyl chloride to 0.20 g (0.97 mmol) of chloro-1-naphthoic acid under Ar. The mixture was heated at reflux for 2 h, cooled to ambient temperature, and the thionyl chloride was removed in vacuo to give the acid chloride, which was added to the indole mixture without further purification. The acid chloride and indole mixture was stirred at ambient temperature for 4 h, quenched with NH_4Cl and extracted with ether. The ethereal solution was allowed to stand at ambient temperature for 20 min upon which time a precipitate was filtered out to give 0.11 g (38%) of 3-(4-chloro-1-naphthoyl)indole as a cream colored solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27–7.31 (m, 2H), 7.52–7.55 (m, 1H), 7.61–7.71 (m, 2H), 7.74–7.76 (m, 2H), 7.80 (d, J = 7.8 Hz, 1H), 8.08 (d, J = 8.1 Hz, 1H), 8.29–8.33 (m, 2H), 12.25 (br s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 112.9, 117.5, 121.9, 122.8, 123.8, 124.5, 126.0, 126.3, 126.6, 128.2, 128.4, 130.5, 131.8, 132.5, 137.5, 137.7, 138.7, 190.8.

5.1.8. 1-Propyl-3-(4-chloro-1-naphthoyl)indole (JWH-400, 10, R = C_3H_7, R′ = H, X = Cl)

A: A solution of 0.16 g (0.52 mmol) of 3-(4-chloro-1-naphthoyl)indole and 0.13 g (2.35 mmol) of crushed KOH in 5 mL of DMSO was stirred at ambient temperature for 1 h. To this mixture was added 0.14 g (0.94 mmol) of **18** and 0.18 g (1.05 mmol) of 1-iodpropane. Stirring at ambient temperature was continued for 6 h. The reaction was quenched with H_2O and the product was extracted with ethyl acetate. After being dried ($MgSO_4$) and concentrated in vacuo the product was purified by column chromatography (petroleum ether/ethyl acetate, 7:3) to give 0.03 g (15%) of JWH-400 as a tan solid: mp 194–195 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.79 (t, J = 7.3 Hz, 3H), 1.73 (sextet, J = 7.3 Hz, 2H), 4.17 (t, J = 7.0 Hz, 2H), 7.33–7.36 (m, 2H), 7.63–7.69 (m, 3H), 7.76 (t, J = 7.5 Hz, 1H), 7.83–8.84 (m, 2H), 8.07 (d, J = 8.0 Hz, 1H), 8.33 (t, J = 8.5 Hz, 2H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 11.4, 23.2, 48.2, 111.6, 116.4, 122.2, 123.1, 123.9, 124.5, 126.1, 126.3, 126.5, 126.8, 128.3, 128.4, 130.6, 131.8, 132.5, 137.4, 138.7, 140.4, 190.4; GC/MS (EI) m/z (rel intensity) 347 (96), 330 (41), 318 (54), 186 (100); HRMS (EI^+) calcd for $C_{22}H_{18}ClNO$: 347.1077; found: 347.0927.

B: JWH-400 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.13 g (0.80 mmol) of 1-propylindole and 0.15 g (0.73 mmol) of 4-chloro-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 7:3) 0.04 g (15%) of 1-propyl-3-(4-bromo-1-naphthoyl)indole (JWH-400) as a tan solid: mp 194–195 °C. The spectroscopic properties were identical to those of material prepared by method A.

5.1.9. 2-Methyl-3-(4-chloro-1-naphthoyl)indole (18, R = CH_3, Ar = 4-chloronaphthyl)

Indole **18** (Ar = 4-chloronaphthyl) was prepared using the procedure employed for the synthesis of 3-(4-chloro-1-naphthoyl)indole. From 0.08 g (0.64 mmol) of 2-methylindole and 4-chloro-1-naphthoyl chloride prepared from 0.12 g (0.58 mmol) of 4-chloro-1-naphthoic acid with 0.29 mL (0.87 mmol) of EtMgBr (3.0 M in diethyl ether]) there was obtained 0.12 g (62%) of 2-methyl-3-(4-chloro-1-naphthoyl)indole as a cream colored solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.95 (t, J = 7.2 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 7.26 (d, J = 7.5 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.46–7.69 (m, 5H), 7.76 (d, J = 7.5 Hz, 2H), 7.87 (d, J = 8.4 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 11.1 (br s, 1H); ^{13}C NMR (75.5 MHz,

DMSO-d_6) δ 14.7, 112.0, 114.0, 120.5, 122.0, 122.6, 124.5, 125.1, 126.0, 126.5, 127.4, 128.3, 128.4, 130.5, 131.2, 132.1, 135.5, 140.7, 146.7, 191.3.

5.1.10. 2-Methyl-1-propyl-3-(4-chloro-1-naphthoyl)indole (JWH-399, 10, R = C3H7, R′ = CH_3, X = Cl)

JWH-399 was prepared by procedure A employed for the synthesis of JWH-400. From 0.12 g (0.37 mmol) of 2-methyl-3-(4-chloro-1-naphthoyl)indole, 0.09 g (1.69 mmol) of crushed KOH and 0.13 g (0.75 mmol) of 1-iodopropane there was obtained after chromatography (petroleum ether/ether; 1:1) 0.06 g (46%) of JWH-399 as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (t, J = 7.2 Hz, 3H), 1.86 (sextet, J = 7.5 Hz, 2H), 2.54 (s, 3H), 4.13 (t, J = 7.2 Hz, 2H), 7.03 (t, J = 7.2 Hz, 1H), 7.16–7.23 (m, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.5 Hz, 2H), 7.63–7.68 (m, 2H), 8.17 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 8.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.5, 12.7, 22.9, 44.9, 109.6, 114.7, 121.1, 122.1, 122.4, 124.7, 125.5, 126.1,126.9, 127.5, 127.7, 131.1, 131.6, 133.7, 136.2, 139.8, 145.9, 192.4; GC/MS (EI) m/z (rel intensity) 361 (100), 346 (26), 326 (56), 304 (29); HRMS (ES^+) m/z calcd for $C_{23}H_{20}ClNO$: 361.1233; found: 361.1232.

5.1.11. 1-Pentyl-3-(4-chloro-1-naphthoyl)indole (JWH-398, 10, R = C_5H_{11}, R′ = H, X = Cl)

JWH-398 was prepared by procedure A employed for the synthesis of JWH-400. From 0.10 g (0.33 mmol) of 3-(4-chloro-1-naphthoyl)indole, 0.08 g (1.5 mmol) of crushed KOH and 0.10 g (0.65 mmol) of 1-bromopentane there was obtained after chromatography (petroleum ether/ether, 9:1) 0.06 g (48%) of JWH-398 as a brown oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 1.28–1.34 (m, 4H), 1.82–1.86 (m, 2H), 4.09 (t, J = 7.0 Hz, 2H), 7.37 (s, 1H), 7.39–7.42 (m, 3H), 7.56 (t, J = 7.5 Hz, 1H), 7.59 (d, J = 7.5 Hz, 1H), 7.65–7.68 (m, 2H), 8.25 (d, J = 8.5 Hz, 1H), 8.40 (d, J = 8.5 Hz, 1H), 8.51–8.53 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.2, 28.9, 29.5, 47.3, 110.1, 117.4, 122.9, 123.0, 123.8, 124.6, 125.0, 125.6, 126.5, 126.9, 127.5, 127.5, 131.0, 132.0, 133.8, 137.1, 138.0, 138.4, 191.0; GC/MS (EI) m/z (rel intensity) 375 (100), 358 (51), 318 (75), 214 (86); HRMS (ES^+) m/z calcd for $C_{24}H_{22}ClNO$: 375.1390; found: 375.1383.

5.1.12. 2-Methyl-1-pentyl-3-(4-chloro-1-naphthoyl)indole (JWH-397, 10, R = C_5H_{11}, R′ = CH_3, X = Cl)

JWH-397 was prepared by procedure A employed for the synthesis of JWH-400. From 0.15 g (0.47 mmol) of 2-methyl-3-(4-chloro-1-naphthoyl)indole, 0.12 g (2.1 mmol) of crushed KOH and 0.14 g (0.94 mmol) of 1-bromopentane there was obtained after chromatography (petroleum ether/ether, 8:2) 0.05 g (31%) of JWH-397 as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (t, J = 6.9 Hz, 3H), 1.37–1.42 (m, 4H), 1.80–1.85 (m, 2H), 2.55 (s, 3H), 4.15 (t, J = 7.5 Hz, 2H), 7.01–7.06 (m, 1H), 7.16–7.24 (m, 2H), 7.35 (d, J = 8.1 Hz, 1H), 7.53 (t, J = 7.2 Hz, 2H), 7.63–7.68 (m, 2H), 8.17 (d, J = 8.4 Hz, 1H), 8.41 (d, J = 8.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.7, 14.0, 22.4, 29.1, 29.3, 43.4, 109.6, 114.7, 121.1, 122.1, 122.4, 124.7, 125.5, 126.2, 127.0, 127.4, 127.7, 131.1, 131.6, 133.7, 136.1, 139.9, 145.9, 192.4; GC/MS (EI) m/z (rel intensity) 389 (100), 374 (81), 354 (43), 332 (30), 304 (27); HRMS (ES^+) m/z calcd for $C_{25}H_{24}ClNO$: 389.1546; found: 389.1541.

5.1.13. 1-Acetyl-4-bromonaphthalene (24, X = Br)

To a stirred solution of 1.00 g (4.8 mmol) of 1-bromonaphthalene in 15 mL of carbon disulfide at 0 °C in a flame-dried flask under N_2 was added 0.42 g (5.3 mmol) of acetyl chloride. This solution was stirred at 0 °C for 10 min and 0.84 g (6.3 mmol) of $AlCl_3$ was added. The reaction was stirred at 0 °C for 3 days followed by 2 days of stirring at ambient temperature. The reaction mixture was poured over ice and concentrated HCl, extracted with ether, washed with $NaHCO_3$ and brine. After drying ($MgSO_4$) the solution was concentrated in vacuo and purified by chromatography (petroleum ether/ether, 95:5) to give 0.75 g (62%) of 1-acetyl-4-bromonaphthalene as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.74 (s, 3H), 7.65–7.69 (m, 2H), 7.74 (d, J = 6 Hz, 1H), 7.83 (d, J = 6 Hz, 1H), 8.32–8.35 (m, 1H), 8.72–8.75 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.1, 126.4, 127.5, 127.8, 128.2, 128.4, 128.7, 128.7, 131.2, 132.3, 135.2, 201.0; GC/MS (EI) m/z (rel intensity) 248 (46), 233 (100), 205 (44). The data agree in all respects with those reported previously.[43]

5.1.14. 4-Bromo-1-naphthoic Acid (22, X = Br)

Acid **22** (X = Br) was prepared by the procedure employed for the conversion of 1-acetyl-4-chloronaphthalene to 4-chloro-1-naphthoic acid. From 0.52 g (2.09 mmol) of 1-acetyl-4-bromonaphthalene there was obtained after chromatography (petroleum ether/ether, 95:5) 0.55 g (57%) of methyl 4-bromo-1-naphthoate as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.00 (s, 3H), 7.63 (p, J = 8.4 Hz, 2H), 7.77 (d, J = 7.5 Hz, 1H), 7.95 (d, J = 7.5 Hz, 1H), 8.30 (d, J = 8.0 Hz, 1H), 8.96 (d, J = 8.0 Hz, 1H) ; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 52.3, 126.3, 126.9, 127.6, 127.7, 128.5, 128.8, 128.9, 130.1, 132.1, 132.4, 167.4; GC/MS (EI) m/z (rel intensity) 264 (65), 233 (98), 295 (35), 126 (100).

Saponification of 0.50 g (1.9 mmol) of methyl-4-bromo-1-naphthoate gave 0.35 g (73%) of 4-bromo-1-naphthoic acid as a white solid: mp 215–217 °C (lit mp 217–219 °C[44]); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.74–7.77 (m, 2H), 8.00 (q, J = 9, 12 Hz, 2H), 8.25–8.28 (m, 1H), 8.90–8.93 (m, 1H), 13.3 (br s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 63.4, 126.7, 127.5, 127.5, 128.6, 129.0, 129.9, 130.5, 131.8, 132.3, 168.6.

5.1.15. 3-(4-Bromo-1-naphthoyl)indole (18, R = H, Ar = 4-bromonaphthyl)

3-(4-Bromo-1-naphthoyl)indole was prepared by the procedure employed for the synthesis of 3-(4-chloro-1-naphthoyl)indole. From 0.14 g (1.2 mmol) of indole and 4-bromo-1-naphthoyl chloride prepared from 0.27 g (1.07 mmol) of 4-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 2:1) 0.29 g (76%) of 3-(4-bromo-1-naphthoyl)indole as a brown solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.27–7.33 (m ,2H), 7.53–7.57 (m, 1H), 7.59–7.64 (m, 2H), 7.71 (d, J = 8.4 Hz, 1H), 7.75–7.80 (m, 1H), 7.98 (d, J = 7.5 Hz, 1H), 8.07 (d, J = 8.1 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.37–8.34 (m, 1H), 12.18 (br, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 113.0, 117.6, 121.9, 122.8, 123.9, 124.0, 126.2, 126.6 (br), 127.3, 128.2, 128.6, 129.7, 131.8, 131.9, 137.5, 137.7, 139.4.

5.1.16. 1-Propyl-3-(4-bromo-1-naphthoyl)indole (JWH-386, 11, R = C_3H_7, R′ = H, X = Br)

A: JWH-386 was prepared by procedure A employed for the synthesis of JWH-400. From 0.30 g (0.86 mmol) of 3-(4-bromo-1-naphthoyl)indole, 0.22 g (3.8 mmol) of crushed KOH and 0.31 g (1.8 mmol) of 1-iodopropane there was obtained after chromatography (petroleum ether/ether, 9:1) 0.040 g (12%) of JWH-386 a white solid: mp 186–188 °C: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.77 (t, J = 7.2 Hz, 3H), 1.72 (sextet, J = 7.2 Hz, 2H), 4.15 (t, J = 6.9 Hz, 2H), 7.32–7.36 (m, 2H), 7.58–7.66 (m, 3H), 7.74 (m, 1H), 7.83 (s, 1H), 8.03 (t, J = 9 Hz, 2H), 8.27 (d, J = 8.4 Hz, 1H), 8.32–8.35 (m, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 11.4, 23.2, 48.1, 111.6, 116.4, 122.2, 123.1, 123.9, 124.0, 126.6, 126.7, 127.2, 128.2, 128.6, 129.8, 131.8, 131.8, 137.4, 139.3, 140.4, 162.3, 190.4; GC/MS (EI) m/z (rel intensity) 391 (64), 376 (22), 364 (23), 207 (100), 186 (67); HRMS (EI^+) m/z calcd for $C_{22}H_{18}BrNO$: 391.0572; found: 391.0590.

B: JWH-386 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.07 g (0.44 mmol) of 1-propylindole and 0.10 g (0.40 mmol) of 4-bromo-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ethyl acetate, 9:1) 0.12 g (72%) of 1-propyl-3-(4-bromo-1-naphthoyl)indole as a yellow solid: mp 186–187 °C. The spectroscopic properties were identical to those of material prepared by method A.

5.1.17. 2-Methyl-3-(4-bromo-1-naphthoyl)indole (18, R = CH_3, Ar = 4-bromonaphthyl)

2-Methyl-3-(4-bromo-1-naphthoyl)indole was prepared by the procedure employed for the synthesis of 3-(4-chloro-1-naphthoyl)indole. From 0.12 g (0.88 mmol) of 2-methylindole and freshly prepared 4-bromo-1-naphthoyl chloride from 0.20 g (0.80 mmol) of 4-bromo-1-naphthoic acid, there was obtained 0.13 g (44%) of 2-methyl-3-(4-bromo-1-naphthoyl)indole as a yellow oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.76 (s , 3H), 6.99 (d, J = 8.0 Hz, 2H), 7.38–7.50 (m, 2H), 7.80 (d, J = 8.0 Hz, 2H), 7.84–7.86 (m, 2H), 8.03 (d, J = 8.0 Hz, 1H), 8.36–8.38 (m, 1H), 12.09 (br, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 15.8, 111.7, 114.0, 120.5, 122.6, 122.8, 125.0, 125.1, 126.0, 126.5, 128.1, 128.5, 129.0, 131.5, 131.7, 132.1, 135.9, 142.4, 148.7, 188.4.

5.1.18. 2-Methyl-1-propyl-3-(4-bromo-1-naphthoyl)indole (JWH-395, 11, R = C_3H_7, R′ = CH_3, X = Br)

A: JWH-395 was prepared by procedure A employed for the synthesis of JWH-400. From 0.22 g (0.60 mmol) of 2-methyl-1-propyl-3-(4-bromo-1-naphthoyl)indole, 0.15 g (2.72 mmol) of crushed KOH and 0.21 g (1.21 mmol) of 1-iodopropane there was obtained after chromatography (petroleum ether/ether, 7:3) 0.12 g (50%) of 2-methyl-1-propyl-3-(4-bromo-1-naphthoyl)indole as a green solid, mp 51–53 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.02 (t, J = 7.5 Hz, 3H), 1.85 (sextet, J = 7.5 Hz, 2H), 2.54 (s, 3H), 4.11 (t, J = 7.5 Hz, 2H), 7.04 (t, J = 7.2 Hz, 1H), 7.14–7.24 (m, 2H), 7.35 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 7.5 Hz, 1H), 7.51 (t, J = 7.2 Hz, 1H), 7.64 (t, J = 7.2 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.38 (d, J = 8.4 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.5, 12.7, 22.9, 44.9, 109.7, 114.7, 121.1, 122.1, 122.4, 124.8, 125.8, 126.2, 126.9, 127.5, 127.7, 127.8, 129.3, 131.6, 132.3, 136.2, 140.7, 146.0, 192.3; GC/MS (EI) *m/z* (rel intensity) 405 (82), 390 (31), 326 (100), 309(24): HRMS (ES^+) *m/z* calcd for $C_{23}H_{20}BrNO$: 405.0728; found: 405.0721.

B: JWH-395 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.08 g (0.48 mmol) of 2-methyl-1-propylindole and 0.11 g (0.45 mmol) of 4-bromo-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ethyl acetate, 8:2) 0.11 g (60%) of 2-methyl-1-propyl-3-(4-bromo-1-naphthoyl)indole as a green oil. The spectroscopic properties were identical to those of material prepared by method A.

5.1.19. 1-Pentyl-3-(4-bromo-1-naphthoyl)indole (JWH-387, 11, R = C_5H_{11}, R′ = H, X = Br)

A: JWH-387 was prepared by procedure A employed for the synthesis of JWH-400. From 0.10 g (0.28 mmol) of 3-(4-bromo-1-naphthoyl)indole, 0.07 g (1.3 mmol) of crushed KOH and 0.09 g (0.57 mmol) of 1-bromopentane there was obtained after chromatography (petroleum ether/ether, 8:2) 0.022 g (18%) of JWH-387 as an orange oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.6 Hz, 3H), 1.25–1.31 (m, 4H), 1.80–1.85 (m, 2H), 4.09 (t, J = 7.2 Hz, 2H), 7.28 (s, 1H), 7.35–7.41 (m, 4H), 7.50–7.56 (m, 1H), 7.63–7.68 (m, 1H), 7.87 (d, J = 7.5 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 8.36 (d, J = 8.4 Hz, 1H), 8.48–8.51 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.2, 28.9, 29.48, 47.2, 110.1, 117.4, 122.9, 123.0, 123.8, 124.8, 125.8, 126.5, 126.9, 127.4, 127.5, 127.8, 128.8, 132.0, 132.2, 137.1, 137.9, 139.1, 191.0; GC/MS (EI) *m/z* (rel intensity) 419 (64), 402 (31), 362 (42), 334 (61), 214 (100); HRMS (EI^+) *m/z* calcd for $C_{24}H_{22}BrNO$: 419.0885; found: 419.0086.

B: JWH-387 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.08 g (0.44 mmol) of 1-pentylindole and 4-bromo-1-naphthoyl chloride prepared from 0.10 g (0.40 mmol) of 4-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 9:1) 0.09 g (53%) of 1-pentyl-3-(4-bromo-1-naphthoyl)indole as an orange oil. The spectroscopic properties of material prepared by method B were identical to those prepared by method A.

5.1.20. 2-Methyl-1-pentyl-3-(4-bromo-1-naphthoyl)indole (JWH-394, 11, R = C_5H_{11}, R′ = CH_3, X = Br

A: JWH-394 was prepared by procedure A employed for the synthesis of JWH-400. From 0.12 g (0.33 mmol) of 2-methyl-3-(4-bromo-1-naphthoyl)indole, 0.08 g (1.48 mmol) of crushed KOH and 0.10 g (0.66 mmol) of 1-bromopentane there was obtained after chromatography (petroleum ether/ether, 8:2) 0.014 g (10%) of 2-methyl-1-pentyl-3-(4-bromo-1-naphthoyl)indole as a green oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (t, J = 7.0 Hz, 3H), 1.39–1.42 (m, 4H), 1.83 (p, J = 7.3 Hz, 2H), 2.55 (s, 3H), 4.15 (t, J = 7.8 Hz, 2H), 7.05 (t, J = 7.3 Hz, 1H), 7.19–7.24 (m, 2H), 7.35 (d, J = 8.0 Hz, 1H), 7.44 (d, J = 7.5 Hz, 1H), 7.52 (t, J = 8.3 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.39 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 12.7, 14.0, 22.4, 29.1, 29.4, 43.4, 109.6, 114.7, 121.1, 122.1, 122.4, 124.8, 125.8, 126.2, 127.0, 127.5, 127.7, 127.8, 129.3, 131.6, 132.3, 136.1, 140.7, 145.9, 192.3; GC/MS (EI) *m/z* (rel intensity) 433 (100), 418 (93), 377 (31), 354 (98), 348 (27), 298 (40), 254 (42), 233 (80); HRMS (EI^+) *m/z* calcd for $C_{25}H_{24}BrNO$: 433.1041; found: 433.1041.

B: JWH-394 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.11 g (0.57 mmol) of 2-methyl-1-pentylindole and 0.13 g (0.52 mmol) of 4-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 7:3) 0.16 g (73%) of 2-methyl-1-pentyl-3-(4-bromo-1-naphthoyl)indole as a green oil. The spectroscopic properties were identical to those of material prepared by method A.

5.1.21. 1-Acetyl-4-iodonaphthalene (24, X = I)

To a stirred solution of 2.00 g (15.4 mmol) of $AlCl_3$ in 10 mL of freshly distilled CH_2Cl_2 under Ar at 0 °C was added dropwise 1.0 g (13.0 mmol) of acetyl chloride. The mixture was stirred at 0 °C for 10 min and 3.0 g (11.2 mmol) of 1-iodonaphthalene was added dropwise over 10 min. The reaction was stirred at 0 °C for 72 h then stirred at ambient temperature for 48 h. The reaction mixture was poured over ice and concentrated HCl, extracted into ether and dried ($MgSO_4$). The solvent was removed in vacuo and the crude product was chromatographed (petroleum ether/ether, 7:3) to give 1.9 g (55%) of 1-acetyl-4-iodonaphthalene as a brown oil, which was converted to the corresponding carboxylic acid without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.71 (s, 3H), 7.49 (d, J = 7.8 Hz, 1H), 7.58–7.64 (m, 2H), 8.08 (d, J = 7.5 Hz, 1H), 8.13–8.17 (m, 1H), 8.63–8.66 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.2, 106.1, 126.5, 128.2, 128.6, 128.8, 130.4, 132.8, 134.6, 136.2, 136.3, 201.3; GC/MS (EI) *m/z* (rel intensity) 296(70), 281 (100), 253 (27), 126 (89). This compound was previously reported by Rajasekaran and Gnanasekaran, however no spectroscopic data were presented.[45]

5.1.22. 4-Iodo-1-naphthoic acid (22, X = I)

Acid **22** (X = I) was prepared by the procedure employed for the conversion of 1-acetyl-4-chloronaphthalene to 4-chloro-1-naphthoic acid. From 2.0 g (6.75 mmol) of 1-acetyl-4-iodonaphthalene

there was obtained after chromatography (petroleum ether/ether, 7:3) 1.2 g (56%) of methyl 4-iodo-1-naphthoate as a brown oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.03 (s, 3H), 7.63–7.69 (m, 2H), 7.83 (d, J = 7.5 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 8.21 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 52.4, 125.8, 126.2, 126.4, 127.8, 128.1, 128.5, 130.3, 133.0, 133.4, 136.5, 167.6; GC/MS (EI) m/z (rel intensity) 312 (100), 281 (84), 253 (22), 126 (76).

Saponification of 1.1 g (3.5 mmol) of methyl-4-iodo-1-naphthoate gave 0.90 g (86%) of 4-iodo-1-naphthoic acid as a white solid: mp 209 °C (lit. mp 213 °C[46]); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72–7.74 (m, 2H), 7.84 (d, J = 8 Hz, 1H), 8.14 (dd, J = 3, 6.5 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H), 8.84 (dd, J = 3, 6.5 Hz, 1H), 13.35 (br s, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 106.1, 126.0, 126.8, 128.8, 128.9, 130.7, 131.5, 132.8, 134.4, 137.2, 168.8.

5.1.23. 1-Propyl-3-(4-iodo-1-naphthoyl)indole (JWH-423, 12, R = C3H7, R′ = H, X = I)

JWH-423 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.11 g (0.70 mmol) of 1-propylindole and 0.21 g (0.70 mmol) of 4-iodo-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ethyl acetate, 9:1) 0.022 g (7%) of 1-propyl-3-(4-iodo-1-naphthoyl)indole as a pale cream powder: mp 160–161 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.79 (t, J = 7 Hz, 3H), 1.72–1.76 (m, 2H), 4.17 (t, J = 7 Hz, 2H), 7.33–7.35 (m, 2H), 7.43 (d, J = 7 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 7.64 (d, J = 7 Hz, 1H), 7.71 (t, J = 7.5 Hz, 1H), 7.81 (s, 1H), 7.98 (d, J = 8 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 8.28 (d, J = 7.5 Hz, 1H), 8.32 (d, J = 6.5 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 11.3, 23.1, 48.9, 101.9, 110.1, 117.5, 122.9, 123.0, 123.8, 126.3, 126.7, 126.9, 127.6, 128.2, 131.4, 132.5, 134.5, 136.4, 137.1, 138.0, 140.1, 191.0; GC/MS (EI) m/z (rel intensity) 439 (100), 422 (37), 410 (40), 186 (83); HRMS (ES^+) m/z calcd for $C_{22}H_{18}INO$: 440.0511; found: 440.0526.

5.1.24. 2-Methyl-1-propyl-3-(4-iodo-1-naphthoyl)indole (JWH-422, 12, R = C_3H_7, R′ = CH_3, X = I)

JWH-422 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.12 g (0.67 mmol) of 2-methyl-1-propylindole and 0.20 g (0.67 mmol) of 4-iodo-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ether, 7:3) 0.039 g (13%) of 2-methyl-1-propyl-3-(4-iodo-1-naphthoyl)indole as a green oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.03 (t, J = 7.5 Hz, 3H), 1.86 (sextet, J = 7.5 Hz, 2H), 2.55 (s, 3H), 4.14 (t, J = 7.5 Hz, 2H), 7.04 (t, J = 8 Hz, 1H), 7.18 (d, J = 8 Hz, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.28 (d, J = 7.5 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.62 (t, J = 7.8 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 7 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 11.5, 12.7, 22.9, 44.9, 101.7, 109.6, 114.7, 121.1, 122.1, 122.4, 126.1, 126.3, 126.9, 127.7, 128.1, 131.0, 132.5, 134.6, 136.2, 136.9, 141.6, 145.9, 192.3; GC/MS (EI) m/z (rel intensity) 453 (100), 326 (58); HRMS (ES^+) m/z calcd for $C_{23}H_{20}INO$: 454.0668; found: 454.0665.

5.1.25. 1-pentyl-3-(4-iodo-1-naphthoyl)indole (JWH-421, 12, R = C_5H_{11}, R′ = H, X = I)

JWH-421 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.13 g (0.67 mmol) of 1-pentylindole and 0.20 g (0.67 mmol) of 4-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 1:1) 0.047 g (15%) of 1-pentyl-3-(4-iodo-1-naphthoyl)indole as a peach colored oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, J = 7.5 Hz, 3H), 1.30–1.36 (m, 4H), 1.83–1.86 (m, 2H), 4.08 (t, J = 7.5 Hz, 2H), 7.35 (s, 1H), 7.36–7.42 (m, 4H), 7.51 (t, J = 8 Hz, 1H), 7.62 (t, J = 8 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 8.19 (d, J = 7 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.48–8.50 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.2, 28.9, 29.5, 47.3, 101.9, 110.1, 117.5, 122.9, 123.0, 123.8, 126.3, 126.7, 126.9, 127.6, 128.2, 131.4, 132.5, 134.5, 136.4, 137.1, 138.0, 140.1, 191.0; GC/MS (EI) m/z (rel intensity) 467 (33), 410 (21), 214 (100), 144 (53); HRMS (ES^+) m/z calcd for $C_{24}H_{22}INO$: 468.0824; found: 468.0811.

5.1.26. 2-Methyl-1-pentyl-3-(4-iodo-1-naphthoyl)indole (JWH-420, 12, R = C_5H_{11}, R′ = CH_3, X = I)

JWH-420 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.10 g (0.50 mmol) of 2-methyl-1-pentylindole and 0.15 g (0.50 mmol) of 4-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 7:3) 0.065 g (27%) of 2-methyl-1-pentyl-3-(4-iodo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (t, J = 6.8 Hz, 3H), 1.39–1.42 (m, 4H), 1.81–1.84 (m, 2H), 2.55 (s, 3H), 4.15 (t, J = 7.5 Hz, 2H), 7.05 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 8 Hz, 1H), 7.22 (t, J = 7.5 Hz, 1H), 7.28–7.29 (m, 1H), 7.35 (d, J = 8 Hz, 1H), 7.50 (t, J = 7.5 Hz, 1H), 7.62 (t, J = 8 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 8.23 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 12.7, 13.9, 22.4, 29.1, 29.3, 43.4, 101.7, 109.6, 114.7, 121.1, 122.2, 122.4, 126.2, 126.3, 126.9, 127.7, 128.1, 131.0, 132.6, 134.6, 136.1, 136.9, 141.6, 145.9, 192.3; GC/MS (EI) m/z (rel intensity) 481 (100), 466 (62), 354 (48), 281 (41); HRMS (ES^+) m/z calcd for $C_{25}H_{24}INO$: 482.0981; found: 482.0987.

5.1.27. 1-Propyl-3-(8-chloro-1-naphthoyl)indole (JWH-456, 14, R = C_3H_7, R′ = H)

JWH-456 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414), however the indole was stirred with dimethylaluminum chloride for 1 h and the acid chloride and indole mixture was stirred for 6 h at ambient temperature. From 0.700 g (3.34 mmol) of 1-propylindole and 0.549 g (2.66 mmol) of 8-chloro-1-naphthoic acid[28] there was obtained after chromatography (petroleum ether/ether, 2:1) 0.425 g (46%) of 1-propyl-3-(8-chloro-1-naphthoyl)indole as a yellow gum: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6 Hz, 3H), 1.75–1.87 (m, 2H), 4.00 (t, J = 6 Hz, 2H), 7.20 (s, 1H), 7.29–7.37 (m, 3H), 7.41(t, J = 9 Hz, 1H), 7.53–7.57 (m, 3H), 7.85 (d, J = 9 Hz, 1H), 7.96 (dd, J = 6 Hz, 3 Hz, 1H), 8.35 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.2, 23.0, 48.7, 109.9, 118.6, 122.5, 122.7, 123.3, 125.4, 126.2, 126.7, 127.5, 127.8, 128.1, 128.7, 129.9, 130.8, 135.6, 137.0, 137.1, 139.0, 192.5; GC/MS (EI) m/z (rel intensity) 347 (97), 312 (100), 270 (42); HRMS (ES^+) m/z calcd for $C_{12}H_{18}ClNO$: 348.1155; found: 348.1161.

5.1.28. 2-Methyl-1-propyl-3-(8-chloro-1-naphthoyl)indole (JWH-461, 14, R = C_3H_7, R′ = CH_3)

JWH-461 was prepared by the procedure employed for the synthesis of 1-propyl-3-(8-chloro-1-naphthoyl)indole (JWH-456). From 0.446 g (2.36 mmol) of 2-methyl-1-propylindole and 0.408 g (1.97 mmol) of 8-chloro-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 3:1) 0.447 g (67%) of 2-methyl-1-propyl-3-(8-chloro-1-naphthoyl)indole as a pale red gum: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95–0.99 (m, 3H), 1.78(s, 3H), 2.97 (s, 1 1/2H), 4.04 (m, 2H), 6.14 (br s, 1/2H), 6.72 (br s, 1/2H), 7.05–7.08 (m, 1H), 7.28 (d, J = 6 Hz, 1H), 7.35–7.40 (m, 1H), 7.44–7.52 (m, 3H), 7.82 (d, J = 6 Hz, 1H), 7.92 (d, J = 6 Hz, 1H), 8.69 (br s, 1/2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.4, 12.5, 22.9, 44.7, 109.5, 114.4, 119.9, 121.5, 122.6, 126.0, 126.1, 126.8, 127.7, 127.8, 128.6, 129.7, 130.7, 134.2, 135.7, 136.0, 140.9, 145.9, 193.6. GC/MS (EI) m/z (rel intensity) 361 (23), 326 (100), 284 (68), 269 (23); HRMS (ES^+) m/z calcd for $C_{23}H_{20}ClNO$: 362.1312; found: 362.1310.

5.1.29. 1-Pentyl-3-(8-chloro-1-naphthoyl)indole (JWH-457, 14, R = C_5H_{11}, R′ = H)

JWH-457 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414), however the indole was stirred with dimethylaluminum chloride for 1 h and the acid chloride and indole mixture was stirred for 6 h at ambient temperature. From 0.780 g (2.90 mmol) of 1-propylindole and 0.507 g (2.42 mmol) of 8-chloro-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 3:1) 0.304 g (33%) of 1-pentyl-3-(8-chloro-1-naphthoyl)indole (JWH-457) as an dark brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.79 (t, J = 6 Hz, 3H), 1.08–1.26 (m, 4H), 1.66–1.75 (m, 2H), 3.94 (t, J = 6 Hz, 2H), 7.15 (s, 1H), 7.26–7.32 (m, 3H), 7.34(m, 1H), 7.45–7.53 (m, 3H), 7.79 (d, J = 9 Hz, 1H), 7.90 (dd, J = 9 Hz, 3 Hz, 1H), 8.39 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.7, 21.9, 28.6, 29.1, 46.8, 109.8, 118.4, 122.4, 122.5, 123.2, 125.3, 126.0, 126.6, 127.4, 127.7, 127.9, 128.6, 129.8, 130.6, 135.4, 136.8, 137.0, 138.8, 192.3; GC/MS (EI) m/z (rel intensity) 375 (80), 340 (100), 270 (48); HRMS (ES^+) m/z calcd for $C_{24}H_{22}ClNO$: 376.1468; found: 376.1459.

5.1.30. 2-Methyl-1-pentyl-3-(8-chloro-1-naphthoyl)indole (JWH-462, 14, R = C_5H_{11}, R′ = CH_3)

JWH-462 was prepared by the procedure employed for the synthesis of 1-propyl-3-(8-chloro-1-naphthoyl)indole (JWH-456). From 0.746 g (3.67 mmol) of 2-methyl-1-pentylindole and 0.632 g (3.06 mmol) of 8-chloro-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ether, 3:1) 0.644 g (54%) of 2-methyl-1-pentyl-3-(8-chloro-1-naphthoyl)indole as a dark brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85–0.88 (m, 3H), 1.31–1.41 (m, 4H), 1.74 (s, 3H), 2.96 (s, 1 1/2H), 3.96–4.07 (m, 2H), 6.15 (br s, 1/2H), 6.71 (br s, 1/2H), 7.03 (m, 1H), 7.26 (d, J = 6 Hz, 1H), 7.33 (t, J = 9 Hz, 1H), 7.44–7.50 (m, 3H), 7.78 (d, J = 6 Hz, 1H), 7.88 (d, J = 6 Hz, 1H), 8.71 (br s, 1/2H). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.2, 13.6, 21.9, 28.6, 28.9, 42.7, 109.3, 113.9, 119.5, 121.3, 122.2, 125.6, 125.8, 126.4, 127.3, 127.5, 128.2, 129.4, 130.2, 135.4, 135.7, 140.6, 144.1, 145.5, 193.1. GC/MS (EI) m/z (rel intensity) 389 (21), 354 (100), 284 (81), 269 (25); HRMS (ES^+) m/z calcd for $C_{25}H_{24}ClNO$: 390.1625; found: 390.1616.

5.1.31. 8-Bromo-1-naphthoic acid (27)

To a mixture of 3.9 mL (67 mmol) of acetic acid and 0.40 mL (22.2 mmol) of H_2O was added with stirring 1.0 g (2.70 mmol) of anhydro-8-hydroxymercuri-1-naphthoic acid[26] and the suspension was stirred at 0 °C for 10 min. A solution of 0.89 g (8.66 mmol) of NaBr in 3.2 mL of water and 0.43 g (2.70 mmol) of bromine were added sequentially and the reaction was slowly heated to 100 °C. After cooling to ambient temperature the mixture was poured into ice and 0.57 g (84%) of 8-bromo-1-naphthoic acid was filtered out as a cream colored solid: mp 173–174 °C (lit. mp 174–175 °C[26]); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 (t, J = 7.8 Hz, 1H), 7.61 (t, J = 7.3 Hz, 1H), 7.68 (d, J = 7.0 Hz, 1H), 7.96 (d, J = 7.3 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 13.32 (s, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 119.3, 126.3, 127.6, 128.0, 128.3, 129.4, 131.2, 133.6, 133.9, 135.7, 171.3.

5.1.32. 1-Propyl-3-(8-bromo-1-naphthoyl)indole (JWH-428, 15, R = C_3H_7, R′ = H)

JWH-428 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.08 g (0.53 mmol) of *N*-propylindole and 0.12 g (0.48 mmol) of 8-bromo-1-naphthoic acid there was obtained after column chromatography (petroleum ether/ethyl acetate, 8:2) 0.09 g (48%) of 1-propyl-3-(8-bromo-1-naphthoyl)indole as an orange solid: mp 101–102 °C; ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.74 (t, J = 7.0 Hz, 3H), 1.71 (q, J = 7.0 Hz, 2H), 4.14–4.18 (m, 2H), 7.27–7.33 (m, 2H), 7.49 (t, J = 8.0 Hz, 1H), 7.61 (t, J = 7.0 Hz, 2H), 7.67 (t, J = 7.5 Hz, 1H), 7.88 (d, J = 7.0 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 11.3, 23.1, 48.0, 111.4, 118.3, 119.6, 122.1, 122.7, 123.5, 126.2, 126.8, 127.4, 128.4, 129.1, 129.5, 130.7, 133.5, 136.1, 137.3, 139.0, 140.0, 191.5: GC/MS (EI) m/z (rel intensity) 391 (10), 312 (100), 270 (99), 254 (26), 241 (25); HRMS (ES^+) m/z calcd for $C_{22}H_{18}BrNO$: 392.0650; found: 392.0637.

At 100 °C: ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.82 (t, J = 7.3 Hz, 3H), 1.78 (sextet, J = 7.3 Hz, 2H), 4.15 (t, J = 7.5 Hz, 2H), 7.24 (t, J = 7.5 Hz, 1H), 7.29 (t, J = 7.5 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 7.56 (d, J = 8.5 Hz, 1H), 7.60 (d, J = 7.0 Hz, 1H), 7.65 (t, J = 7.5 Hz, 1H), 7.86 (d, J = 7.5 Hz, 1H), 8.09 (d, J = 8.5 Hz, 1H), 8.13 (t, J = 7.8 Hz, 2H).

5.1.33. 2-Methyl-1-propyl-3-(8-bromo-1-naphthoyl)indole (JWH-429, 15, R = C3H7, R′ = CH_3)

JWH-429 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.13 g (0.66 mmol) of 2-methyl-1-propylindole and 0.15 g (0.60 mmol) of 8-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 8:2) 0.04 g (16%) of 2-methyl-1-propyl-3-(8-bromo-1-naphthoyl)indole as a green oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.81–0.90 (m, 3H), 1.65–1.76 (m, 2H), 2.89 (s, 1 1/2H), 4.23–4.24 (m, 2H), 6.63–6.66 (m, 1/2H), 7.01–7.03 (m, 1/2H), 7.26–7.27 (m, 1/2H), 7.43–7.54 (m, 2 1/2H), 7.64 (t, J = 7.5 Hz, 1H), 7.83–7.91 (m, 1H), 8.11–8.17 (m, 2H), 8.39 (br s, 1/2H); (At 100 °C: ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.89 (t, J = 7.5 Hz, 3H), 1.76 (sextet, J = 7.3 Hz, 2H), 2.89 (br s, 3H), 4.17 (t, J = 7.0 Hz, 2H), 6.92 (br s, 1H), 7.13 (t, J = 7.5 Hz, 1H), 7.45 (t, J = 7.5 Hz, 3H), 7.63 (t, J = 7.5 Hz, 1H), 7.83 (d, J = 7.5 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 11.5, 14.3, 23.0, 44.7, 109.5, 120.0, 120.2, 121.4, 121.6, 122.7, 125.9, 126.6, 127.4, 127.7, 128.6, 129.2, 130.16, 132.9, 136.1, 142.2, 144.4, 146.0, 193.5. GC/MS (EI) m/z (rel intensity) 405 (10), 326 (100), 284 (79), 269 (31); HRMS (ES^+) m/z calcd for $C_{23}H_{20}BrNO$: 406.0807; found: 406.0788.

5.1.34. 1-Pentyl-3-(8-bromo-1-naphthoyl)indole (JWH-424, 15, R = C_5H_{11}, R′ = H)

JWH-424 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.12 g (0.66 mmol) of *N*-pentylindole and 0.15 g (0.60 mmol) of 8-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 7:3) 0.20 g (78%) of *N*-pentyl-3-(8-bromo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.86 (t, J = 7.0 Hz, 3H), 1.22–1.32 (m, 4H), 1.77–1.81 (m, 2H), 4.00–4.02 (m, 2H), 7.19–7.29 (m, 1H), 7.31–7.43 (m, 4H), 7.53 (t, J = 7.5 Hz, 1H), 7.62 (d, J = 7.0 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.2, 28.8, 29.4, 47.1, 110.1, 119.3, 120.2, 122.7, 122.8, 123.5, 125.4, 126.7, 126.9, 128.1, 128.7, 129.5, 130.4, 133.1, 136.0, 137.1, 137.3, 140.1, 192.3; GC/MS (EI) m/z (rel intensity) 421 (3), 340 (100), 270 (58); HRMS (ES^+) m/z calcd for $C_{24}H_{22}BrNO$: 420.0963; found: 420.0959.

5.1.35. 2-Methyl-1-pentyl-3-(8-bromo-1-naphthoyl)indole (JWH-425, 15, R = C_5H_{11}, R′ = CH_3)

JWH-425 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.13 g (0.66 mmol) of 2-methyl-1-pentylindole and 0.15 g (0.60 mmol) of 8-bromo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ethyl acetate, 9:1) 0.04 g (15%) of 2-methyl-1-pentyl-3-(8-bromo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ

0.83–0.88 (m, 3H), 1.25–1.34 (m, 4H), 1.64–1.77 (m, 2H), 2.91 (s, 1 1/2H), 4.21–4.27 (m, 2H), 5.89 (br s, 1/2H), 6.67 (br s, 1/2H), 7.04 (br s, 1/2H), 7.27 (br s, 1H), 7.48–7.52 (m, 2 ½H), 7.65 (t, J = 7.5 Hz, 1H), 7.86–7.91 (m, 1H), 8.13–8.17 (m, 1 1/2H), 8.41 (br s, 1/2H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 12.7, 14.0, 22.4, 29.1, 29.4, 43.4, 109.7, 120.1, 121.4, 121.7, 122.7, 125.9, 126.6, 127.4, 127.5, 128.6, 130.1, 132.9, 133.4, 135.4, 136.1, 142.2, 144.3, 146.0, 193.5. GC/MS (EI) m/z (rel intensity) 433 (6), 354 (78), 284 (100), 269 (28); HRMS (ES$^+$) m/z calcd for $C_{25}H_{24}BrNO$: 434.1120; found: 434.1128.

5.1.36. 8-Iodo-1-naphthoic acid (28)

To 5.00 g (13.5 mmol) of anhydro-8-(hydroxymercuri)-1-naphthoic acid was added 9.63 g (58.0 mmol) of potassium iodide in 47 mL of H_2O. After dissolution, 3.42 g (13.5 mmol) of iodine was added and the reaction mixture was heated at reflux for 15 h. The solution was cooled to ambient temperature and a brown precipitate was filtered out. To the filtrate was added 1.62 g (10 mmol) of sodium thiosulfate in 9 mL of H_2O to destroy excess iodine and the solution was acidified by the dropwise addition of concd HCl. The crude acid was filtered out, dissolved in hot acetone and concentrated *in vacuo*. Recrystallization from $CHCl_3$ gave 2.1 g (52%) of 8-iodo-1-naphthoic acid as a white solid: mp 155–156 °C (lit. mp 164–165°C[26]); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (t, J = 7.8 Hz, 1H), 7.51 (t, J = 7.5 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 2H), 8.27 (d, J = 7.5 Hz, 1H), 12.63 (br s, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 93.1, 125.1, 127.5, 129.5, 129.6, 131.4, 132.6, 133.5, 135.4, 141.9, 175.5.

5.1.37. 1-Propyl-3-(8-iodo-1-naphthoyl)indole (JWH-419, 16, R = C_3H_7, R′ = H)

JWH-419 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.11 g (0.69 mmol) of 1-propylindole and 0.31 g (1.0 mmol) of 8-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 1:1) 0.064 g (21%) of *N*-propyl-3-(8-iodo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (Ambient temperature, 500 MHz, DMSO-d_6) δ 0.78 (t, J = 7.5 Hz, 3H), 1.73 (q, J = 7.0 Hz, 2H), 4.15–4.22 (m, 2H), 7.28–7.33 (m, 3H), 7.62–7.65 (m, 3 1/2H), 8.11–8.14 (m, 2H), 8.23 (d, J = 7.0 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 11.3, 23.1, 48.0, 94.1, 111.3, 119.3, 122.3, 122.3, 122.7, 123.5, 125.9, 126.9, 127.8, 128.7, 130.0, 131.2, 131.9, 135.8, 137.3, 141.8, 141.9, 190.9; GC/MS (EI) m/z (rel intensity) 439 (2), 312 (100), 270 (63); HRMS (ES$^+$) m/z calcd for $C_{22}H_{18}INO$: 440.0511; found: 440.0491.

5.1.38. 2-Methyl-1-propyl-3-(8-iodo-1-naphthoyl)indole (JWH-418, 16, R = C_3H_7, R′ = CH_3)

JWH-418 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.14 g (0.84 mmol) of 2-methyl-1-propylindole and 0.36 g (1.2 mmol) of 8-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 3:7) 0.13 g (35%) of 2-methyl-1-propyl-3-(8-iodo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.87–0.95 (m, 3H), 1.70–1.86 (m, 2H), 2.91 (s, 1 1/2H), 4.16–4.27 (m, 2H), 5.99–6.00 (m, 1/2H), 6.69–6.72 (m, 1/2H), 7.03–7.06 (m, 1/2H), 7.28–7.31 (m, 1 1/2H), 7.44–7.46 (m, 1H), 7.54–7.62 (m, 2H), 8.12–8.15 (m, 2H), 8.20–8.28 (m, 1H), 8.42 (br s, 1/2H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 11.5, 13.0, 23.0, 44.9, 92.9, 109.3, 109.7, 116.0, 120.5, 121.4, 121.7, 122.7, 125.7, 127.1, 128.0, 129.5, 130.7, 132.0, 135.9, 141.4, 143.8, 146.2, 192.8; GC/MS (EI) m/z (rel intensity) 453 (6), 326 (100), 284 (95), 269 (54), 254 (31); HRMS (ES$^+$) m/z calcd for $C_{23}H_{20}INO$: 454.0668; found: 454.0662.

5.1.39. 1-Pentyl-3-(8-iodo-1-naphthoyl)indole (JWH-416, 16, R = C_5H_{11}, R′ = H)

JWH-416 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.10 g (0.57 mmol) of 1-pentylindole and 0.23 g (0.77 mmol) of 8-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 1:1) 0.040 g (11%) of 1-pentyl-3-(8-iodo-1-naphthoyl)indole as a yellow oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.88 (t, J = 7.0 Hz, 3H), 1.26–1.32 (m, 4H), 1.81–1.83 (m, 2H), 4.04–4.14 (m, 2H), 7.20 (t, J = 7.0 Hz, 1H), 7.38–7.43 (m, 2 1/2H), 7.54 (t, J = 7.0 Hz, 1H), 7.67 (d, J = 7.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 2H), 8.22 (d, J = 7.5 Hz, 1H); ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 13.9, 22.2, 28.8, 29.4, 47.1, 93.2, 109.9, 122.7, 123.0, 123.5, 125.1, 126.9, 127.0, 127.3, 128.5, 129.1, 129.5, 130.9, 132.0, 132.2, 135.7, 137.1, 141.6, 191.6; GC/MS (EI) m/z (rel intensity) 341 (100), 324 (48), 284 (86), 214 (85), 144 (48); HRMS (EI$^+$) calcd for $C_{24}H_{22}INO$: 467.0747; found: 467.0742.

5.1.40. 2-Methyl-1-pentyl-3-(8-iodo-1-naphthoyl)indole (JWH-417, 16, R = C_5H_{11}, R′ = CH_3)

JWH-417 was prepared by the procedure employed for the synthesis of 1-propyl-3-(4-fluoro-1-naphthoyl)indole (JWH-414). From 0.17 g (0.84 mmol) of 2-methyl-1-pentylindole and 0.25 g (0.84 mmol) of 8-iodo-1-naphthoic acid there was obtained after chromatography (petroleum ether/ether, 1:1) 0.25 g (64%) of 2-methyl-1-pentyl-3-(8-iodo-1-naphthoyl)indole as a brown oil: ^{1}H NMR (Ambient Temperature, 500 MHz, DMSO-d_6) δ 0.84–0.89 (m, 3H), 1.24–1.36 (m, 4H), 1.65–1.84 (m, 2H), 2.91 (s, 1 1/2H), 4.17–4.28 (m, 2H), 5.99–6.01 (m, 1/2H), 6.69–6.71 (m, 1/2H), 7.03–7.06 (m, 1/2H), 7.28–7.31 (m, 2H), 7.45–7.48 (m, 1H), 7.52–7.61 (m, 2H), 8.13–8.26 (m, 3H), 8.43 (br s, 1/2H); ^{13}C NMR (125.77 MHz, DMSO-d_6) δ 13.0, 14.3, 22.3, 28.8, 29.4, 43.1, 93.8, 110.7, 119.7, 121.5, 122.0, 122.6, 123.0, 126.4, 127.9, 130.0, 131.1, 131.6, 135.8, 136.2, 141.7, 144.0, 146.3, 191.8; GC/MS (EI) m/z (rel intensity) 481 (3), 354 (70), 284 (100), 269 (45), 254 (28); HRMS (ES$^+$) m/z calcd for $C_{25}H_{24}INO$: 482.0981; found: 482.0961.

5.2. Receptor binding experiments

5.2.1. Materials

All chemicals were from Sigma (St. Louis, MO) except the following: [^{35}S]GTPγS (1250 Ci/mmol) was purchased from New England Nuclear Group (Boston, MA), GTPγS from Boehringer Mannheim (New York, NY), and DMEM/F-12 from Fischer Scientific (Pittsburg, PA). Whatman GF/B glass fiber filters were purchased from Fisher Scientific (Pittsburgh, PA).

5.2.2. Membrane preparations

Human embryonic kidney-293 cells stably expressing the human CB_1 receptor were cultured in DMEM with 10% fetal bovine serum, and Chinese hamster ovary cells stably expressing the human CB_2 receptor were cultured in DMEM with 10% fetal calf serum. Cells were harvested by replacement of the medium with ice-cold phosphate-buffered saline containing 1 mM EDTA followed by centrifugation at 1000g for 5 min at 4 °C. The pellet was resuspended in 50 mM Tris–HCl containing 320 mM sucrose, 2 mM EDTA, and 5 mM $MgCl_2$, pH 7.4 (centrifugation buffer), and then centrifuged at 1000g for 10 min at 4 °C, and the resulting supernatant was saved. This process was repeated twice. The supernatant fractions were combined and centrifuged at 40,000g for 30 min at 4 °C. The resulting P2 pellet was resuspended in assay buffer (50 mM Tris–HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl), and protein was measured. Membranes were stored at −80 °C until use.

5.2.3. Receptor binding

Membrane homogenates (50 μg) were incubated with 0.5 nM [^{3}H]CP 55,940 in the presence of varying concentrations (1 nM–10 μM) of test compounds in 0.5 mL of buffer containing bovine serum albumin (5 mg/mL). Nonspecific binding was measured in the presence of 1 μM CP 55,940. The assay was incubated at 30 °C for 1 h and terminated by the addition of ice-cold 50 mM Tris-HCl containing bovine serum albumin (1 mg/mL, pH 7.4) followed by filtration under vacuum through Whatman GF/B glass fiber filters with three washes with ice-cold Tris buffer. Bound radioactivity was determined by liquid scintillation spectrophotometry at 50% efficiency after extraction by shaking samples for 30–60 min with Budget–Solve scintillation fluid.

5.3. [35S]GTPγS binding

5.3.1. [^{35}S]GTPγS binding assay

Prior to assays, samples were thawed on ice, centrifuged at 50,000*g* for 10 min at 4 °C, and resuspended in Assay Buffer (50 mM Tris–HCl (pH 7.4), 3 mM $MgCl_2$, 0.2 mM EGTA, and 100 mM NaCl). Concentration–effect curves were generated by incubating membranes (10 μg) in assay buffer and bovine serum albumin (1 mg/mL) with various concentrations of test compounds in the presence of 20 μM GDP and 0.1 nM [^{35}S]GTPγS in a 1 mL of total volume. [^{35}S]GTPγS binding stimulated by 2 μM CP55,940 was used as an internal standard in each assay. Basal binding was assessed in the absence of agonist, and nonspecific binding was measured in the presence of 10 μM GTPγS. The reaction was incubated for 90 min at 30 °C and terminated by filtration under vacuum through Whatman GF/B glass fiber filters with three washes with ice-cold (4 °C) Tris buffer (50 mM Tris–HCl, pH 7.4). Bound radioactivity was determined by liquid scintillation spectrophotometry at 95% efficiency after extraction overnight in Scinti-Safe Econo 1 scintillation fluid (Fisher Scientific, Hampton, NH).

5.3.2. Data analysis

All in vitro data are reported as the means ± SEM of at least three experiments, each performed in triplicate. For the receptor binding experiments, displacement IC_{50} values were originally determined by Hill plots and then converted to K_i values using the method of Cheng and Prusoff.[47] For the [^{35}S]GTPγS binding experiment, nonlinear regression analysis was conducted to obtain EC_{50} and E_{max} values of agonist-stimulated [^{35}S]GTPγS binding by iterative curve fitting with JMP (SAS, Cary, NC).

5.4. Pharmacology

5.4.1. Subjects

Male ICR mice (Harlan Laboratories, Dublin, VA) weighing 18–25 g were maintained on a 14:10 hr light:dark cycle with free access to food and water. Δ^9-THC was obtained from the National Institute on Drug Abuse. All compounds were dissolved in 1:1:18 (emulphor–ethanol–saline) for in vivo administration. Emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ) is currently available as Alkmulphor. All drug injections were administered iv (tail vein) at a volume of 0.1 mL/10 g of body weight. Following drug administration each animal was tested for effects on the following procedures: spontaneous (locomotor) activity at 5–15 min, tail-flick latency (antinociception) response at 20 min, core (rectal) temperature at 30 min and ring immobility (catalepsy) at 40–45 min. Separate mice were tested with each dose of a compound (n = 6 per dose).

5.4.2. Spontaneous activity

Measurement of locomotor activity was accomplished by placing mice into individual activity cages (6.5 × 11 in), and recording interruptions of the photocell beams (16 beams per chamber) for a 10-min period using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, OH). Activity in the chamber was recorded as the total number of beam interruptions and was expressed as % inhibition of the level of activity of the vehicle group.

5.4.3. Tail-flick latency

Antinociception was assessed using the tail-flick procedure. The heat lamp of the tail-flick apparatus was maintained at an intensity sufficient to produce control latencies of 2–3 s. Control values for each animal were determined prior to drug administration. Mice were then re-evaluated following drug administration and latency (s) to tail-flick response was recorded. A 10-sec maximum was imposed to prevent tissue damage. The degree of antinociception was expressed as the % MPE (maximum possible effect) which was calculated as:

$$\%\mathrm{MPE} = \left[\frac{(\text{test latency} - \text{control latency})}{(10\ \text{s} - \text{test latency})}\right]$$

5.4.4. Core temperature

Hypothermia was assessed by first measuring baseline core temperatures prior to drug treatment with a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH) and a rectal thermistor probe inserted to 25 mm. Rectal temperatures were also measured following drug administration. Rectal temperature values were expressed as the difference between control temperature (before injection) and temperatures following drug administration (Δ°C).

5.4.5. Catalepsy

Catalepsy was determined by using a slight modification of the ring test as developed by Pertwee.[48] Mice were injected iv with either vehicle or cannabinoid and 40 min after treatment were placed on a ring (5.5 cm in diameter) which was attached to a ring stand and raised to a height of 16 cm. Mice were rated for catalepsy by observers who were blind with regard to treatment. During a 5-min test session, the amount of time (sec) for which the mouse was motionless (except for respiratory movements) was determined. Mice that either fell or actively jumped from the ring were allowed 5 such 'escapes.' If these occurred before 2.5 min, the animal's data were disregarded. An immobility index was determined by dividing the amount of time spent motionless by the length of the test session (300 s) and multiplying by 100 to determine an immobility index for each mouse.

5.4.6. Data analysis

When supply of a compound was sufficient to evaluate multiple doses, potency was determined based on a scheme we have used in numerous previous studies with cannabinoids, with maximal cannabinoid effects in each procedure estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail flick procedure, −6 °C change in rectal temperature, and 60% ring immobility. ED_{50} was defined as the dose at which half maximal effect occurred. For compounds that produced one or more cannabinoid effect, ED_{50} was calculated separately using least-squares linear regression on the linear part of the dose–effect curve for each measure in the mouse tetrad, plotted against $\log_{10}$ transformation of the dose. When limited supply prevented assessment of potency, a probe dose of the compound was tested the in vivo test battery. In these cases, the test dose is specified in the appropriate table and values obtained in each test are reported directly as % inhibition of locomotion, % maximal antinociceptive effect, change in rectal temperature, and % ring immobility.

Acknowledgments

The work at Clemson was supported by Grant DA03590 to J.W.H. and that at the Research Triangle Institute by Grants DA-031988 and DA-03672 to J.L.W., all from the National Institute on Drug Abuse.

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