1-Benzhydryl-3-phenylurea and 1-Benzhydryl-3-phenylthiourea Derivatives: New Templates among the CB_1 Cannabinoid Receptor Inverse Agonists

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New 1-benzhydryl-3-phenylurea derivatives and their 1-benzhydryl-3-phenylthiourea isosteres were synthesized and evaluated for their human CB_1 and CB_2 cannabinoid receptor affinity. These compounds proved to be selective CB_1 cannabinoid receptor ligands, acting as inverse agonists in a [^{35}S]-GTPγS assay. The affinity of 3,5,5′-triphenylimidazolidine-2,4-dione and 3,5,5′-triphenyl-2-thioxoimidazolidin-4-one derivatives, possessing the 1-benzhydryl-3-phenylurea and 1-benzhydryl-3-phenylthiourea moiety, respectively, was also evaluated. In conclusion, the 1-benzhydryl-3-phenylurea scaffold seems to be a new interesting template of CB_1 cannabinoid receptor inverse agonists.

Introduction

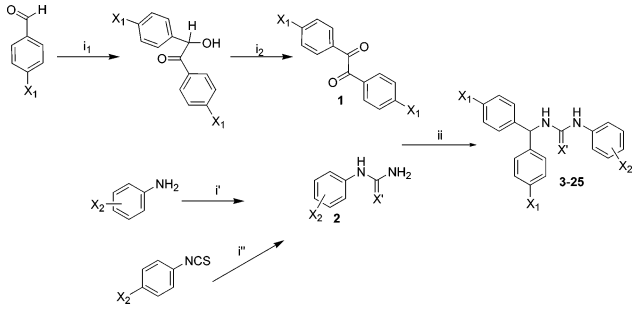
The synthesis of the first potent CB_1 cannabinoid receptor antagonist/inverse agonist SR141716A[1] (rimonabant) and the breeding of knock-out CB_1 mice[2,3] confirmed the high therapeutic potential for modulating CB_1 cannabinoid receptor activity. Several CB_1 cannabinoid receptor inverse agonists were shown to reduce food intake,[4,5] ethanol consumption,[6] and nicotine reward[7,8] in animals. Other studies suggested a beneficial effect on septic shock in rats[9] and in gastrointestinal disorders.[10] Subsequent phase II or III clinical trials confirmed the usefulness of such compounds in treating obesity[11,12] and nicotine[11] addictions. Among the cannabinoid receptor antagonists/inverse agonists available, most are constructed around a central heterocyclic ring (pyrazole, triazole, indole, imidazolidinedione, or thioxoimidazolidinone) bearing further substituted aromatic rings.[13]

In this paper, we report the CB_1 cannabinoid receptor inverse agonist properties of new compounds that are not built around such a central ring. The 1-benzhydryl-3-phenylurea derivatives described herein exhibited interesting affinities and potencies for the CB_1 cannabinoid receptor. The 1-benzhydryl-3-phenylthiourea isosteres also showed affinity and inverse agonist properties at the CB_1 cannabinoid receptor. The compounds were then compared with the corresponding 3-phenyl-5,5′-diphenylimidazolidine-2,4-dione and 3-phenyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives.

Results and Discussion

Chemistry. Benzoin condensation starting from substituted benzhaldehydes was used for the synthesis of the substituted benzils (**1**). Phenylurea (**2**, X′ = O) and phenylthiourea (**2**, X′ = S) derivatives were easily synthesized from the corresponding aniline and phenylisothiocyanate, respectively (Scheme 1). Target 1-benzhydryl-3-phenylurea derivatives (**3**−**21**) were obtained by reaction of the respective benzil (**1**) and phenylurea (**2**, X′ = O), following a microwave-enhanced method previously described[14] (Scheme 1). The corresponding thio derivatives (**22**−**25**) were obtained similarly by reacting **1** and phenylthiourea (**2**, X′ = S). The synthesis of the thio derivatives, reported here for the first time, will allow the oxo and thio isostere comparison at the CB_1 and CB_2 cannabinoid receptors. The 3,5,5′-triphenylimidazolidine-2,4-dione (**26**−**29**) and 3,5,5′-triphenyl-2-thioxoimidazolidin-4-one derivatives (**30**, **31**) were obtained by reaction of **1** and phenylurea (**2**, X′ = O) or phenylthiourea (**2**, X′ = S) in ethanol in the presence of ethanolate (Scheme 2).

Scheme 1[a]



[a] All X_2 substituents are in the para position except that for **8** (X_2 is in ortho). Reagents and conditions: (i_1) H_2O/EtOH, NaCN, reflux 3 h, $CHCl_3$ extraction of the oily benzoin intermediate; (i_2) HNO_3, reflux, 2 h; (i′) CH_3COOH/H_2O, NaCNO, room temp; (i″) acetone, NH_4OH, room temp; (ii) DMSO/aqueous KOH, nine microwave pulses.

Pharmacology. An initial screening, performed at 10 μM, using human CB_1 and CB_2 cannabinoid receptors expressed in Chinese hamster ovarian (CHO) cells

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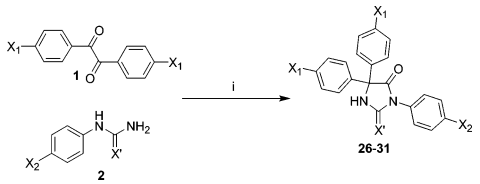
Scheme 2[a]

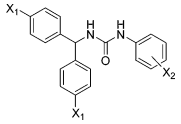
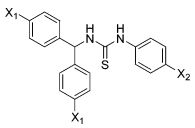
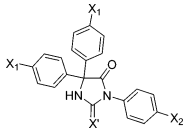


[a] Reagents and conditions: EtOH, Na, reflux 12 h.

and either [^{3}H]-SR141716 or [^{3}H]-CP-55,940 as radioligands for the CB_1 or CB_2 cannabinoid receptors, respectively, allowed the establishment of some preliminary structure−affinity relationships (Table 1). First, the benzhydryl moiety must be substituted to achieve CB_1 cannabinoid receptor affinity. Indeed compounds **3**, **4**, and **6**−**9** displaced less than 25% of [^{3}H]-SR141716A specific binding. Furthermore, the preferred benzhydryl substitutents are chlorine (**12**) and bromine (**13**). Second, comparing the radioligand displacements obtained with **14**−**21** revealed that chlorine and bromine are the preferred substituents for the phenyl moiety as well. Compounds **3**−**21** showed only poor displacement of the CB_2 cannabinoid receptor bound radioligand ([^{3}H]-CP-55,940). K_i values of **12**−**18**, which showed the highest radioligand displacement at 10 μM at the CB_1 cannabinoid receptor, were obtained (Table 1). Substitution of the phenyl moiety (X_2 in Scheme 1) greatly increased the affinity for the CB_1 cannabinoid receptor as illustrated by **12** ($K_i = 7050 \pm 550$ nM) and **14** ($K_i = 1100 \pm 100$ nM) or by **13** ($K_i = 2900 \pm 250$ nM) and **16** ($K_i = 650 \pm 50$ nM). With respect to the bromobenzhydryl derivatives (**13**, **16**, **17**, **19**−**21**), the bromine substituent (X_2 in Scheme 1) proved to be the preferred substituent at the para position of the phenyl ring (**17**, $K_i = 500 \pm 50$ nM). Smaller and larger halogen atoms resulted in decreased affinity, as illustrated by **16** ($K_i = 650 \pm 50$ nM) and **19** ($32 \pm 2\%$ of displacement at 10 μM), respectively. Replacing the bromobenzhydryl (X_1 in Scheme 1) by a chlorobenzhydryl (**15**, $K_i = 1250 \pm 100$ nM) or an iodobenzhydryl (**18**, $K_i = 750 \pm 100$ nM) moiety also decreased the affinity. Four 1-benzhydryl-3-phenylthioureas (**22**−**25**) were obtained to estimate the effect of the oxygen−sulfur substitution (Table 1). The affinity of the unsubstituted 3-phenyl derivatives was increased by the replacement of the oxygen atom by the sulfur as illustrated by **12** ($K_i = 7050 \pm 550$ nM) and **22** ($K_i = 2900 \pm 250$ nM) or by **13** ($K_i = 2900 \pm 250$ nM) and **23** ($K_i = 1800 \pm 150$ nM). However, the affinity of the substituted 3-phenyl derivatives (X_2 in Scheme 1) is decreased for the thio derivatives as shown by **14** ($K_i = 1100 \pm 100$ nM) and **24** ($K_i = 1900 \pm 200$ nM) or by **16** ($K_i = 650 \pm 50$ nM) and **25** ($K_i = 1500 \pm 150$ nM). Thus, replacement of the oxygen by a sulfur atom in 1-[bis(4-bromophenyl)methyl]-3-(4-chlorophenyl)urea (**16**) resulted in decreased affinity.

We previously described the structure−affinity relationships at the CB_1 cannabinoid receptor of alkyl and alkylaryl 3-substituted 5,5′-diphenylimidazolidine-2,4-diones[15] and 5,5′-diphenyl-2-thioxoimidazolidin-4-ones[16] as selective CB_1 cannabinoid receptor inverse agonists. Therefore, four 3,5,5′-triphenylimidazolidine-2,4-diones (**26**−**29**) and two 3,5,5′-triphenyl-2-thioxoimidazolidin-4-one derivatives (**30**, **31**) were synthesized (Scheme 2) and assayed for their CB_1 cannabinoid receptor affinity (Table 1). The four imidazolidine-2,4-diones assayed at

Table 1. Percentage Displacement of [^{3}H]-SR141716A and [^{3}H]-CP-55,940 Specific Binding (10 μM) and Affinities (K_i, If Given) for **3**−**31**[a] on hCB_1 and hCB_2 Cannabinoid Receptors[b]

				% displacement		
compd	X′	X_1	X_2	hCB_1 receptor	hCB_2 receptor	hCB_1 K_i (nM)
						
3	O	H	H	<20	<10	ND
4	O	H	F	23 ± 2	<10	ND
5	O	H	Cl	43 ± 2	<20	ND
6	O	H	Br	<20	<20	ND
7	O	H	I	<10	<10	ND
8	O	H	2-F	<20	<10	ND
9	O	H	OMe	<20	<20	ND
10	O	H	C_6H_{13}	55 ± 2	24 ± 2	ND
11	O	F	H	<20	<10	ND
12	O	Cl	H	70 ± 3	23 ± 2	7050 ± 550
13	O	Br	H	70 ± 2	38 ± 3	2900 ± 250
14	O	Cl	Cl	95 ± 1	24 ± 2	1100 ± 100
15	O	Cl	Br	80 ± 2	29 ± 3	1250 ± 100
16	O	Br	Cl	83 ± 1	31 ± 2	650 ± 50
17	O	Br	Br	75 ± 3	22 ± 2	500 ± 50
18	O	I	Br	60 ± 2	19 ± 2	750 ± 100
19	O	Br	I	32 ± 2	34 ± 1	ND
20	O	Br	CH_2OH	49 ± 2	<20	ND
21	O	Br	C_6H_{13}	<20	<20	ND
						
22	S	Cl	H	77 ± 4	23 ± 2	2900 ± 250
23	S	Br	H	86 ± 2	29 ± 3	1800 ± 150
24	S	Cl	Cl	85 ± 1	23 ± 2	1900 ± 200
25	S	Br	Cl	71 ± 3	29 ± 4	1500 ± 150
						
26	O	H	H	28 ± 3	< 20	ND
27	O	H	Br	< 20	< 20	ND
28	O	F	H	27 ± 3	< 20	ND
29	O	Br	H	29 ± 4	< 20	ND
30	S	Br	H	73 ± 2	<20	3450 ± 300
31	S	Br	Cl	62 ± 2	<20	2750 ± 250
			Reference Compounds			
SR141716A				ND	ND	5.4 ± 0.2
WIN-552122				ND	ND	3800 ± 150
HU-210				ND	ND	19 ± 2

[a] All X_2 substituents are in the para position except that for **8** (X_2 is in ortho). [b] Mean ± SEM of at least four experiments performed in duplicate. The K_i values were obtained from nonlinear analysis of competition curves using [^{3}H]-SR141716A as radioligand.

10 μM, 3,5,5′-triphenylimidazolidine-2,4-dione (**26**), 3-(4-bromophenyl)-5,5′-diphenylimidazolidine-2,4-dione (**27**), 5,5′-bis(4-fluorophenyl)-3-phenylimidazolidine-2,4-dione (**28**), and 5,5′-bis(4-bromophenyl)-3-phenylimidazolidine-2,4-dione (**29**), displaced less than 30% of the [^{3}H]-SR141716A specifically bound to the CB_1 cannabinoid receptor. The 2-thioxoimidazolidin-4-ones, 5,5′-bis(4-bromophenyl)-3-phenyl-2-thioxoimidazolidin-4-one (**30**) and 5,5′-bis(4-bromophenyl)-3-(4-chlorophenyl)-2-thioxoimidazolidin-4-one (**31**), exhibited K_i values of 3450 ± 300 and 2750 ± 250 nM, respectively. When these values are compared with those obtained for the corre-

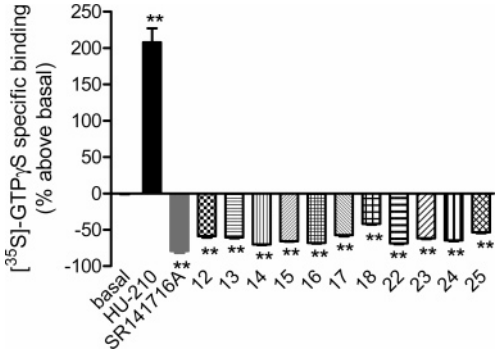


Figure 1. [^{35}S]-GTPγS binding stimulation assay of selected compounds and reference cannabinoid ligands (10 μM) on hCB$_1$ cannabinoid receptor. Data are the mean ± SEM of at least three experiments performed in duplicate. Statistical significance was assessed by one-way ANOVA followed by a Dunett post-test: (**) $P < 0.01$.

Table 2. Determination of the Potency (EC_{50}) and Percentages of Basal Maximal Stimulation (E_{max}) of [^{35}S]-GTPγS Binding at hCB$_1$ Cannabinoid Receptors for **14**−**17**, **25**, HU-210, and SR141716A[a]

compd	EC_{50} (nM)	E_{max} (% above basal)
	Urea Derivatives	
14	1050 ± 100	−74 ± 2
15	700 ± 50	−66 ± 1
16	450 ± 50	−72 ± 2
17	500 ± 50	−61 ± 1
	Thiourea Derivatives	
25	1250 ± 100	−61 ± 3
	Reference Compounds	
SR141716A	10.1 ± 0.7	−84 ± 2
HU-210	0.6 ± 0.04	200 ± 15

[a] Mean ± SEM of at least three experiments performed in duplicate. Statistical signification of [^{35}S]-GTPγS assay results was assessed using a one-way ANOVA followed by a Dunett post-test.

sponding 1-benzhydryl-3-phenylurea and thiourea derivatives, it appears that the orientation of the 1-benzhydryl-3-phenylurea and thiourea phenyl rings imposed by the additional carbonyl led to a decrease in the affinity for the CB$_1$ cannabinoid receptor. This is illustrated by **13** and **29** (70% and 29% of displacement at 10 μM, respectively) or by **25** and **31** (K_i of 1500 ± 150 and 2750 ± 250 nM, respectively).

The 1-benzhydryl-3-phenylurea and thiourea derivatives were further characterized in a functional [^{35}S]-GTPγS assay as previously described.[17] The assay relies on the binding of [^{35}S]-GTPγS, a radiolabeled nonhydrolyzable GTP analogue, to the G protein upon activation by an agonist of the G-protein-coupled receptor.[18] The assay distinguishes between agonists (increasing nucleotide binding), antagonists (not affecting the binding), and inverse agonists (decreasing nucleotide binding). HU-210, a potent cannabinoid agonist, the inverse agonist SR141716A, and the 1-benzhydryl-3-phenylurea and thiourea derivatives **12**−**18** and **22**−**25** were screened at 10 μM. The cannabinoid receptor agonist HU-210 induced an increase in the [^{35}S]-GTPγS binding (200 ± 15% compared to basal level), while SR141716A and the tested compounds significantly decreased the nucleotide binding, thus acting as inverse agonists of the human CB$_1$ cannabinoid receptor (Figure 1). For instance, 1-[bis(4-bromophenyl)methyl]-3-(4-chlorophenyl)urea (**16**) and its thio derivative 1-[bis(4-bromophenyl)methyl]-3-(4-chlorophenyl)thiourea (**25**) decreased [^{35}S]-GTPγS binding by 68 ± 1% and 61 ± 1% compared to basal level, respectively. To further explore the inverse agonist properties, the potency of **14**−**17** and **25** was determined (Table 2). The 1-benzhydryl-3-phenylureas **14**−**17** and the thiourea derivative **25** dose-dependently decreased the [^{35}S]-GTPγS binding, showing EC_{50} values of the same magnitude compared to their respective K_i values.

In conclusion, the 1-benzhydryl-3-phenylurea and 1-benzhydryl-3-phenylthiourea derivatives reported here selectively bind to the CB$_1$ cannabinoid receptor acting as inverse agonists. The structure−affinity relationships highlighted the importance of halogen substituents, especially bromine. Thus, 1-[bis(4-bromophenyl)methyl]-3-(4-bromophenyl)urea (**17**) exhibited the highest affinity for the CB$_1$ cannabinoid receptor within this series of compounds. Interestingly, the crucial role of halogen substitution of the phenyl rings is also observed in the structure−affinity relationships of SR141716A and other CB$_1$ cannabinoid receptor inverse agonists.[13] Finally, despite a lower affinity compared to the affinity of the reference inverse agonist SR141716A, the 1-benzhydryl-3-phenylurea structure constitutes a new, interesting template for CB$_1$ cannabinoid receptor inverse agonists. Indeed the noncyclic core scaffold CB$_1$ cannabinoid receptor inverse agonist described herein differs from most of the reported CB$_1$ cannabinoid receptor antagonists/inverse agonists, which are built around a cyclic central unit.

Experimental Section

All reagents, used without further purification, were purchased from Sigma-Aldrich or Acros. Solvents were of analytical grade. A commercial household microwave oven (2.45 Ghz) was used. Melting points (uncorrected) were determined in open capillaries using an Electrothermal 9100 apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer at room temperature and analyzed using WIN-NMR software. Chemical shifts (δ) are reported relative to the tetramethylsilane peak set at 0.00 ppm. Signals were abbreviated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. For multiplets, signals are reported as intervals. Coupling constants are expressed in hertz. Mass spectra were recorded on a Finnigan MAT 44S, with an ionization voltage of 70 eV. Elemental analyses were performed on a Carlo Erba EA 1108 analyzer (Carlo Erba, Milano, Italy) and are within ±0.4% of the theoretical values. Infrared (IR) spectra (ν in cm^{-1}) were recorded on a Perkin-Elmer FT-IR 286 spectrometer (Supporting Information).

Synthesis: **General Procedure for the Synthesis of 1-Benzhydryl-3-phenylurea Derivatives.** Compounds **3**, **5**, **7**, **11**−**13**, **15**, **17**, and **19** were previously described.[14] The other 1-benzhydryl-3-phenylurea derivatives were similarly obtained. To a solution of benzil (7.15 mmol) and phenylurea (14.3 mmol) in 25 mL of DMSO was added with stirring 25 mL of 1.2 M aqueous KOH. Following 90 s of 750 W microwave irradiation, the mixture was stirred for an additional 5 min. Additional 30 s pulses were applied at 6, 9, 12, 15, 18, 21, 24, and 30 min. Between pulses, the mixture was stirred. After the completion of the sequence, the mixture was poured onto cold water and the resulting precipitate was filtered, dried, and crystallized. All microwave irradiations were carried out in an open system.

1-Benzhydryl-3-(4-fluorophenyl)urea (4). Yield: 52%. Mp 229.1−230.2 °C. MS DEI (Desorption Electron Impact): 320 [M]$^+$. ^{13}C NMR (DMSO-d_6) δ 56.71 (CH), 114.88, 115.20, 119.02, 126.78, 127.43, 128.34, 136.43, 143.02 (C and CH arom), 154.21 (C=O). Anal. ($C_{20}H_{17}FN_2O$) C, H, N.

1-Benzhydryl-3-(4-bromophenyl)urea (6). Yield: 50%. Mp 233.4−234.1 °C. MS DEI: 381 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 56.97 (CH), 112.68, 119.67, 127.04, 128.59, 131.57, 143.16 (C and CH arom), 154.28 (C=O). Anal. ($C_{20}H_{17}BrN_2O$) C, H, N.

1-Benzhydryl-3-(2-fluorophenyl)urea (8). Yield: 55%. Mp 229.5−230.2 °C. MS DEI: 320 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 56.87 (CH), 114.58, 114.84, 119.69, 121.44, 121.57, 124.35, 126.81, 126.94, 128.43, 142.99 (C and CH arom), 153.01 (C=O), 153.92.

1-Benzhydryl-3-(4-methoxyphenyl)urea (9). Yield: 51%. Mp 215.0−215.9°C. MS DEI: 332 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 55.06 (CH_3), 56.74 (CH), 113.87, 119.11, 126.81, 128.36, 133.28, 143.25 (C and CH arom), 153.92 (C=O), 154.44. Anal. ($C_{21}H_{20}N_2O_2$) C, H, N.

1-Benzhydryl-3-(4-hexylphenyl)urea (10). Yield: 48%. Mp 172.1−173.2 °C. MS DEI: 386 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 13.84, 21.99, 28.14, 31.06, 34.35, and 40.12 (CH_2), 56.74 (CH), 117.56, 126.81, 128.36, 135.05, 137.74, 143.18 (C and CH arom), 154.31 (C=O). Anal. ($C_{26}H_{30}N_2O$) C, H, N.

1-[Bis(4-chlorophenyl)methyl]-3-(4-chlorophenyl)-urea (14). Yield: 46%. Mp >300 °C. MS DEI: 406 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 56.20 (CH), 119.60, 120.70, 125.29, 128.99, 129.70, 131.89, 139.47, 142.38 (C and CH arom), 154.54 (C=O). Anal. ($C_{20}H_{15}Cl_3N_2O$) C, H, N.

1-[Bis(4-bromophenyl)methyl]-3-(4-chlorophenyl)-urea (16). Yield: 49%. Mp >300 °C. MS DEI: 494 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 55.81 (CH), 119.34, 124.97, 128.21, 128.66, 131.90, 139.20, 141.73 (C and CH arom), 154.28 (C=O). Anal. ($C_{20}H_{15}Br_2ClN_2O$) C, H, N.

1-[Bis(4-iodophenyl)methyl]-3-(4-bromophenyl)urea (18). Yield: 43%. Mp 246.1−247.0 °C. MS DEI: 633 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 56.13 (CH), 93.20, 112.74, 119.73, 129.43, 131.38, 131.51, 137.39, 137.65, 139.59, 142.51 (C and CH arom), 154.21 (C=O). Anal. ($C_{20}H_{15}I_2BrN_2O$) C, H, N.

1-[Bis(4-bromophenyl)methyl]-3-(4-hydroxymethylphenyl)urea (20). Yield: 52%. Mp 213.4−214.3 °C. MS DEI: 490 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 56.19 (CH), 60.98 (CH_2), 120.31, 121.86, 127.49, 128.07, 129.44, 131.57, 137.91, 142.38 (C and CH arom), 154.67 (C=O). Anal. ($C_{21}H_{18}Br_2N_2O_2$*1/2H_2O) C, H, N.

1-[Bis(4-bromophenyl)methyl]-3-(4-hexylphenyl)-urea (21). Yield: 49%. Mp 202.8−203.3 °C. MS DEI: 544 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 14.08, 18.67, 22.17, 28.37, 31.16, and 34.59 (CH_2), 55.87 (CH), 117.92, 120.31, 128.53, 129.31, 131.57, 135.39, 137.78, 142.31 (C and CH arom), 154.47 (C=O). Anal. ($C_{26}H_{28}Br_2N_2O$) C, H, N.

Synthesis of 1-Benzhydryl-3-phenylthiourea Derivatives. The procedure described for the synthesis of 1-benzhydryl-3-phenylurea derivatives was used except that phenylthiourea was substituted for phenylurea.

1-[Bis(4-chlorophenyl)methyl]-3-phenylthiourea (22). Yield: 43%. Mp 185.1−185.8 °C. MS DEI: 387 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 59.23 (CH), 122.64, 123.94, 128.27, 128.40, 129.11, 131.76, 139.34, 140.50 (C and CH arom), 180.23 (C=S). Anal. ($C_{20}H_{16}Cl_2N_2S$) C, H, N.

1-[Bis(4-bromophenyl)methyl]-3-phenylthiourea (23). Yield: 44%. Mp 206.8−207.5 °C. MS DEI: 476 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 59.37 (CH), 120.25, 122.64, 123.94, 128.27, 129.44, 131.31, 139.34, 140.89 (C and CH arom), 180.23 (C=S). Anal. ($C_{20}H_{16}Br_2N_2S$) C, H, N.

1-[Bis(4-chlorophenyl)methyl]-3-(4-chlorophenyl)thiourea (24). Yield: 41%. Mp 200.8−201.4 °C. MS DEI: 422 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 59.30 (CH), 119.03, 124.20, 127.69, 128.40, 129.12, 131.77, 138.36, 140.37 (C and CH arom), 180.22 (C=S). Anal. ($C_{20}H_{15}Cl_3N_2S$) C, H, N.

1-[Bis(4-bromophenyl)methyl]-3-(4-chlorophenyl)thiourea (25). Yield: 43%. Mp 230.0−230.3 °C. MS DEI: 511 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 59.43 (CH), 120.31, 124.20, 127.69, 128.07, 129.05, 131.31, 138.30, 140.67 (C and CH arom), 180.22 (C=S). Anal. ($C_{20}H_{15}Br_2ClN_2S$) C, H, N.

General Procedure for the Synthesis of 3-Substituted 5,5′-Diphenylimidazolidine-2,4-dione Derivatives. Sodium (1 g) was stirred in ethanol (50 mL) for 15 min before benzil (9.5 mmol) and phenylurea (19 mmol) were added. The resulting solution was refluxed for 12 h and after cooling was poured onto ice. The precipitate was filtered, dried, and recrystallized from ethanol.

3,5,5′-Triphenylimidazolidine-2,4-dione (26). Yield: 62%. Mp 204.2−204.9 °C. MS DCI: 329 $[M + H]^+$. ^{13}C NMR (DMSO-d_6): δ 69.21 (C), 117.66, 121.24, 126.97, 128.33, 128.59, 128.79, 129.04, 131.82, 139.66 (C and CH arom), 154.28 (C=O), 172.46 (C=O).

3-(4-Bromophenyl)-5,5′-diphenylimidazolidine-2,4-dione (27). Yield: 58%. Mp 214.4−215.1 °C. MS DEI: 408 $[M + H]^+$. ^{13}C NMR (DMSO-d_6): δ 69.65 (C), 121.54, 127.23, 128.79, 129.11, 129.24, 131.44, 132.35, 139.86 (C and CH arom), 154.22 (C=O), 172.52 (C=O). Anal. ($C_{21}H_{15}Br_2N_2O_2$) C, H, N.

5,5′-Bis(4-fluorophenyl)-3-phenylimidazolidine-2,4-dione (28). Yield: 57%. Mp 242.9−244.0 °C. MS DEI: 364 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 68.03 (C), 115.27, 115.6, 126.72, 128.07, 128.72, 128.85, 131.44, 135.45, 160.11, 163.34 (C and CH arom), 153.83 (C=O), 172.01 (C=O). Anal. ($C_{21}H_{14}F_2N_2O_2$) C, H, N.

5,5′-Bis(4-bromophenyl)-3-phenylimidazolidine-2,4-dione (29). Yield: 58%. Mp 162.5−163.7 °C. MS DEI: 486 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 68.45 (C), 121.94, 126.56, 128.46, 128.96, 129.54, 129.87, 131.78, 138.45 (C and CH arom), 154.05 (C=O), 171.76 (C=O). Anal. ($C_{21}H_{14}Br_2N_2O_2$) C,H,N.

Synthesis of 3-Substituted 5,5′-Diphenyl-2-thioxoimidazolidin-4-one Derivatives. These derivatives were obtained similarly to the 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives starting from the corresponding phenylthiourea.

5,5′-Bis(4-bromophenyl)-3-phenyl-2-thioxoimidazolidin-4-one (30). Yield: 40%. Mp 293.0−293.9 °C. MS DEI: 502 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 70.89 (C), 122.26, 128.79, 128.98, 131.83, 132.67, 136.94 (C and CH arom), 172.33 (C=O), 181.00 (C=S). Anal. ($C_{21}H_{14}Br_2N_2OS$) C, H, N.

5,5′-Bis(4-bromophenyl)-3-(4-chlorophenyl)-2-thioxoimidazolidin-4-one (31). Yield: 41%. Mp >300 °C. MS DEI: 536 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 71.53 (C), 122.83, 129.37, 131.18, 132.02, 132.41, 134.22, 137.40 (C and CH arom), 172.72 (C=O), 181.19 (C=S). Anal. ($C_{21}H_{13}Br_2ClN_2OS$) C, H, N.

Competition Binding Assay. CHO cells stably expressing the human CB_1 or the human CB_2 cannabinoid receptors were donated by Drs. M. Detheux and P. Nokin, respectively (Euroscreen s.a., Gosselies, Belgium). Cells membranes were obtained as previously described.[16] Stock solutions of the compounds were prepared in DMSO and further diluted (100×) with the binding buffer to the desired concentration. Under these conditions B_{max} was 57 pmol/mg protein and K_d was 1.13 ± 0.13 nM for the hCB_1 cannabinoid receptor ([^{3}H]-SR141716A). The competitive binding experiments were performed using [^{3}H]-SR141716A (1 nM, 52 Ci/mol, Amersham, Roosendaal, The Netherlands) or [^{3}H]-CP-55940 (1 nM, 101 Ci/mol, from NEN Life Science, Zaventem, Belgium) as radioligands for the hCB_1 and the hCB_2 cannabinoid receptor, respectively, at 30 °C in plastic tubes, and 40 μg of membranes per tube was resuspended in 0.5 mL (final volume) of binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). Test compounds were present at varying concentrations, and nonspecific binding was determined in the presence of 10 μM HU-210 (Tocris, Bristol, U.K.). After 1 h of incubation, solutions were rapidly filtered through 0.5% PEI pretreated GF/B glass fiber filters (Whatman, Maidstone, U.K.) on a M-48T Brandell cell harvester and washed twice with 5 mL of ice-cold binding buffer without serum albumin. Radioactivity was measured in a Pharmacia Wallac 1410 β-counter in 10 mL of Aqualuma (PerkinElmer, Schaesberg, The Netherlands) after 10 s of shaking and 3 h of resting. Assays were performed at least in triplicate.

[^{35}S]-GTPγS Assay. Binding experiments were performed at 30 °C in plastic tubes containing 40 μg of protein in 0.5 mL (final volume) of binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, 0.1% bovine serum albumin, pH 7.4) supplemented with 20 μM GDP and the appropriate

concentration of test compounds. The assay was initiated by the addition of [^{35}S]-GTPγS (0.05 nM, final concentration, 1173 Ci/mmol, Amersham, Roosendaal, The Netherlands). Following 1 h of incubation, a total of 5 mL of ice-cold washing buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl) was added. The suspension was immediately filtered through GF/B filters using a 48-well Brandell cell harvester and washed twice with the same ice-cold buffer. Radioactivity was counted as mentioned above. Nonspecific binding was measured in the presence of 100 μM Gpp(NH)p. Assays were performed in triplicate.

Data Analysis. IC_{50} and EC_{50} values were determined by nonlinear regression analysis performed using the GraphPad Prism 4.0 software (GraphPad Software, San Diego). The K_i values were calculated on the basis of the Cheng−Prusoff equation: $K_i = IC_{50}/(1 + L/K_d)$.

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Supporting Information Available: General procedures for the synthesis of benzils, phenylureas, and phenylthioureas, ^{1}H NMR and IR data, and results from elemental analysis of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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