Substituted 2-Thioxoimidazolidin-4-ones and Imidazolidine-2,4-diones as Fatty Acid Amide Hydrolase Inhibitors Templates

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The demonstration of the essential role of fatty acid amide hydrolase (FAAH) in hydrolyzing endogenous bioactive fatty acid derivatives has launched the quest for the discovery of inhibitors for this enzyme. Along this line, a set of 58 imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives was evaluated as FAAH inhibitors. Among these compounds, 3-substituted 5,5′-diphenylimidazolidine-2,4-dione and 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives were previously described as CB_1 cannabinoid receptor ligands. In the present study, we synthesized several derivatives exhibiting interesting FAAH inhibitory activity and devoid of affinity for the CB_1 and CB_2 cannabinoid receptors. For instance, 3-heptyl-5,5′-diphenylimidazolidine-2,4-dione (**14**) and 5,5′-diphenyl-3-tetradecyl-2-thioxo-imidazolidin-4-one (**46**) showed pI_{50} values of 5.12 and 5.94, respectively. In conclusion, it appears that even though several 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one and 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives have been previously shown to behave as CB_1 cannabinoid receptor ligands, appropriate substitutions of these templates can result in FAAH inhibitors devoid of affinity for the cannabinoid receptors.

Introduction

The cloning of the first cannabinoid receptor,[1] named CB_1, in 1990 was rapidly followed by the report of the discovery of its first endogenous ligand, arachidonoylethanolamide,[2] or anandamide (AEA), which acts as a partial agonist.[3,4] Nowadays, several other compounds are considered as endocannabinoids, i.e. endogenous ligands of the cannabinoid receptors. Among them, 2-arachidonoylglycerol[5,6] (2-AG), the most abundant endocannabinoid in the brain,[7] is a full CB_1 cannabinoid receptor agonist.[8] The characterization of anandamide and other endocannabinoids launched the quest for the discovery of the enzyme(s) responsible(s) for their degradation. For anandamide, the question was partially answered with the characterization of an enzyme, now widely called fatty acid amide hydrolase[9−11] (FAAH). This membrane-bound enzyme was cloned[12] in 1996 and crystallized[13] in 2002, by Cravatt's group. FAAH is responsible for the hydrolysis of arachidonoylethanolamide to yield arachidonic acid and ethanolamine. Later on, 2-AG was shown to be a substrate for FAAH, at least in vitro.[14,15] Moreover, FAAH is also involved in the hydrolysis of other bioactive fatty acid amides, such as the sleep-inducing agent oleamide,[16,17] the anorexigenic compound *N*-oleoylethanolamide[18] (OEA), and the antiinflammatory agent *N*-palmitoylethanolamide[15] (PEA).

Since its discovery, several pharmacological effects were reported for anandamide. Among these were antinociception,[19] modulation of anxiety,[20] and control of reproduction.[21] In addition, mice lacking FAAH showed elevated anandamide levels[22] as well as a CB_1 cannabinoid receptor-dependent analgesia phenotype.[23] Thus, inhibition of fatty acid amide hydrolase, enhancing fatty acid amides effects, is considered as a very promising therapeutic target.[24]

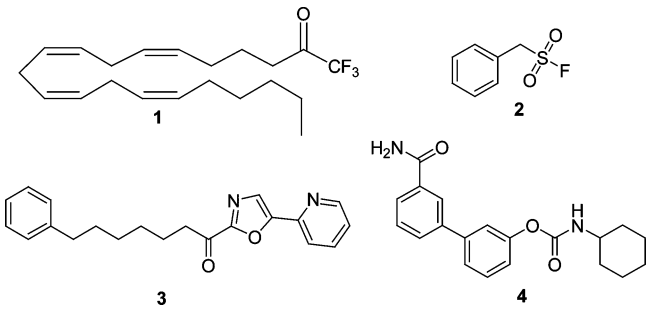

Figure 1. Structures of four FAAH inhibitors: ATMFK (**1**), PMSF (**2**), OL-135 (**3**), and URB-597 (**4**).

An increasing number of compounds were described as FAAH inhibitors (Figure 1).[25] Among them are, for instance, arachidonoyl trifluoromethyl ketone (**1**, ATFMK) and phenylmethylsulfonyl fluoride (**2**, PMSF). Interestingly, more recent inhibitors, such as OL-135[26,27] (**3**) or URB-597[20] (**4**), induce analgesia after in vivo administration. OL-135, as well as the recent 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles,[28] were shown to be highly selective for FAAH over other serine hydrolase enzymes using a proteome screening approach.[29] The therapeutic potential of FAAH inhibitors, e.g. to treat pain and anxiety, further highlights the interest in the synthesis and characterization of new FAAH inhibitors.

In this connection, we have found in a preliminary screening assay that some 3-substituted 5,5′-diphenylimidazolidine-2,4-dione are able to inhibit FAAH activity. The 3-substituted 5,5′-diphenylimidazolidines-2,4-diones[30,31] and their thioxo analogues the 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-

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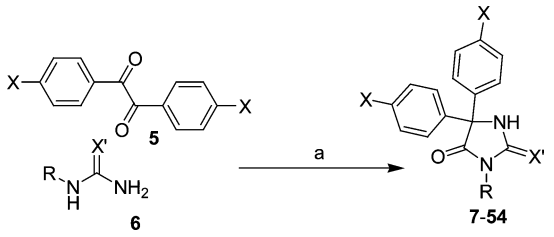
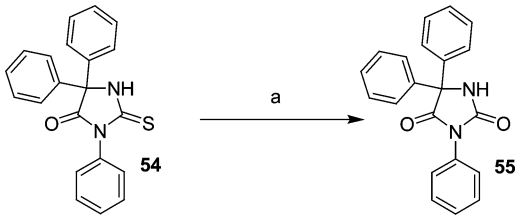
Scheme 1. Synthesis of the 5,5′-Diphenylimidazolidine-2,4-dione (X′ = O, **7**−**28**) and 5,5′-Diphenyl-2-thioxoimidazolidin-4-one (X′ = S, **29**−**54**) Derivatives[a]



[a] Reagents and conditions: (a) DMSO/aq KOH, nine microwaves pulses (750 W).

Scheme 2. Synthesis of the 3,5,5′-Triphenylimidazolidine-2,4-dione (**55**)[a]



[a] Reagents and conditions: (a) stirred for 24 h in DMF/CH_3COOH at rt in the presence of H_2O_2.

one[32] derivatives were previously described as CB_1 cannabinoid receptor ligands. In this paper, we report the ability of such compounds to inhibit FAAH. Preliminary structure−activity relationships, as well as the inhibition mode, are discussed. These derivatives represent new interesting FAAH reversible inhibitors.

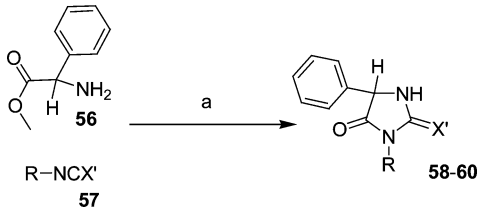
Results and Discussion

Chemistry. 5,5′-Diphenylimidazolidine-2,4-dione and 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives were synthesized from the respective benzil (**5**) and urea (**6**, X′ = O) or thiourea (**6**, X′ = S) derivatives using a microwave-enhanced method previously described[33] (Scheme 1). The method allowed the rapid synthesis of the target derivatives (**7**−**54**) in moderate to good yields. However, when phenylurea is used, the resulting compound is 1-benzhydryl-3-phenylurea instead of the target 3-substituted imidazolidine-2,4-dione.[34] Therefore, 3,5,5′-triphenylimidazolidine-2,4-dione (**55**) was obtained, in high yield, from the corresponding 2-thioxoimidazolidin-4-one (**54**) upon reaction with hydrogen peroxide in DMF−acetic acid (Scheme 2). This reaction was already used for *N*-alkyl derivatives[33]and has been extended to the *N*-phenyl derivatives.

3-Substituted 5-phenylimidazolidine-2,4-dione (**58**) and 3-substituted 5-phenyl-2-thioxoimidazolidin-4-one (**59** and **60**) derivatives were synthesized in two steps by reacting phenylglycine methyl ester (**56**) with the desired phenyl or alkyl isocyanate/isothiocyanate (**57**, X′ = O or S), respectively (Scheme 3). The first step consisted of the condensation of the amino acid derivative with a phenyl or alkyl iso(thio)cyanate in pyridine, leading to 3-substituted (thio)ureido-phenyl acetic acid. The second step allowed the cyclization of the acid derivative upon refluxing in acidic water.

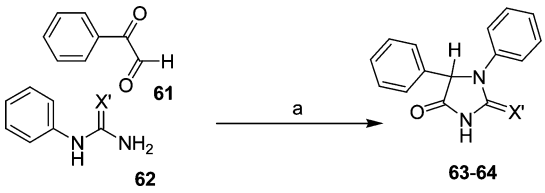
1,5-Diphenylimidazolidine-2,4-dione (**63**) and 1,5-diphenyl-2-thioxoimidazolidin-4-one (**64**) were obtained, as previously described by Joshi et al.,[35] by reacting phenylglyoxal (**61**) with phenylurea (**62**, X′ = O) and phenylthiourea (**62**, X′ = S), respectively (Scheme 4). The synthesis, carried out in glacial

Scheme 3. Synthesis of the 3-Substituted 5-Phenylimidazolidine-2,4-dione (X′ = O, **58**) and 2-Thioxoimidazolidin-4-one Derivatives (X′=S, **59** and **60**)[a]



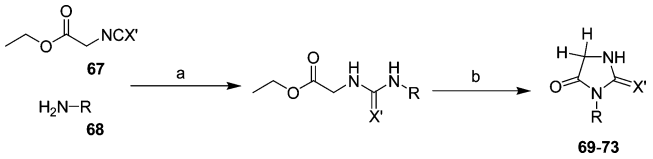
[a] Reagents and conditions: (a) stirred for 24 h in pyridine at 40 °C. After evaporation of pyridine, the residue is refluxed in 2 N HCl.

Scheme 4. Synthesis of the 1,5-Diphenyl Derivatives (**63** and **64**)[a]



[a] Reagents and conditions: (a) refluxed for 4 h in CH_3COOH/HCl.

Scheme 5. Synthesis of the 3-Substituted Imidazolidine-2,4-dione (X′ = O, **69**−**71**) and 2-Thioxoimidazolidin-4-one (X′ = S, **72** and **73**) Derivatives[a]



[a] Reagents and conditions: (a) stirred for 4 h in $CHCl_3$; (b) following evaporation of $CHCl_3$, refluxed for 3 h in ethanol and HCl (10 N) (1:1, v/v).

acetic acid with hydrochloric acid as catalyst, allowed the synthesis of both compounds in good yields.

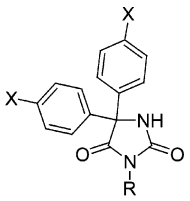
3-Heptyl-1,5-diphenylimidazolidine-2,4-dione (**65**) and 1,3-diheptyl-5,5′-diphenylimidazolidine-2,4-dione (**66**) were obtained by alkylation in DMF of the corresponding 3-unsubstituted derivatives (**63** and **14**, respectively) using chloroheptane.

The compounds bearing no substituent in position 5 of the central moiety were obtained following a method described by Ryczek[36] (Scheme 5). Glycine ethyl ester isocyanate (**67**, X′ = O) and the desired amine (**68**) were refluxed in chloroform to afford the 3-substituted ureidoacetic acid ethyl ester, which upon refluxing in ethanol/hydrochloric acid cyclized to form the imidazolidine-2,4-dione nucleus bearing the desired substituent in position 3 (**69**−**71**). Following the same procedure, but starting from glycine ethyl ester isothiocyanate (**67**, X′ = S) the 3-substitituted imidazolidine-2,4-dione derivatives (**72**, **73**) were obtained in good yields.

FAAH Inhibition and Primary Structure−Activity Relationships. An initial screening, performed at 10 μM, using rat brain homogenates as source of FAAH and [^{3}H]-anandamide as substrate, revealed that some 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives inhibited FAAH activity. Therefore, a set of 21 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives and a set of 26 thioxo analogues have been challenged in a FAAH inhibition assay, to establish structure−activity relationships.

The results, expressed as pI_{50} values, obtained for the 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives are summarized in Table 1 (pI_{50} = −log IC_{50}). The influence

Table 1. 3-Substituted 5,5′-Diphenylimidazolidine-2,4-dione Derivatives (**7**−**28**, **55**)[a]



compd	X	R	pI_{50}	% of displacement hCB$_1$ receptor	% of displacement hCB$_2$ receptor
7	H	H	<2	<20	<20
8	H	C_2H_5	3.76 ± 0.02	<20	<20
9	H	C_4H_9	4.29 ± 0.02	<20[b]	<20[b]
10	Cl	C_4H_9	4.99 ± 0.03	51.2 ± 7.1	<20
11	Br	C_4H_9	4.71 ± 0.09	69.2 ± 6.5	23.1 ± 5.4
12	H	C_5H_{11}	4.53 ± 0.03	21.3 ± 5.5[b]	<20[b]
13	H	C_6H_{13}	5.02 ± 0.02	29.9 ± 4.5[b]	<20[b]
14	H	C_7H_{15}	5.12 ± 0.03	23.4 ± 5.7	<20
15	Cl	C_7H_{15}	4.31 ± 0.15	42.7 ± 5.1	<20
16	Br	C_7H_{15}	4.56 ± 0.12	44.8 ± 6.6[b]	<20[b]
17	H	C_8H_{17}	4.87 ± 0.03	25.1 ± 5.2[b]	<20[b]
18	F	C_8H_{17}	4.89 ± 0.05	40.1 ± 4.3[b]	<20[b]
19	Cl	C_8H_{17}	3.62 ± 0.05	39.5 ± 6.4	<20
20	Br	C_8H_{17}	3.30 ± 0.04	45.1 ± 5.0[b]	21.1 ± 4.2[b]
21	Me	C_8H_{17}	3.79 ± 0.04	24.5 ± 4.3	<20
22	OMe	C_8H_{17}	3.52 ± 0.10	31.2 ± 5.3	<20
23	H	$C_{10}H_{21}$	4.31 ± 0.08	<20	<20
24	H	$C_{14}H_{29}$	3.98 ± 0.11	<20	<20
25	H	C_6H_{11}	3.88 ± 0.17	24.3 ± 6.5	<20
55	H	C_6H_5	4.13 ± 0.05	28.5 ± 6.6	<20
26	H	$CH_2C_6H_5$	4.73 ± 0.05	33.7 ± 6.1	<20
27	Cl	$CH_2C_6H_5$	5.00 ± 0.12	72.2 ± 6.8	<20
28	H	$(CH_2)_2C_6H_5$	4.30 ± 0.07	<20	<20

[a] Inhibition potential toward FAAH (rat brain homogenate, pI_{50} ± SEM) and affinity for the hCB$_1$ and hCB$_2$ cannabinoid receptor expressed in CHO cells (% of displacement of bound [^{3}H]-SR141716A and [^{3}H]-CP-55,940, for the hCB$_1$ and hCB$_2$ cannabinoid receptor, respectively, obtained with 10 μM of competitor, mean ± SEM). [b] From ref 30.

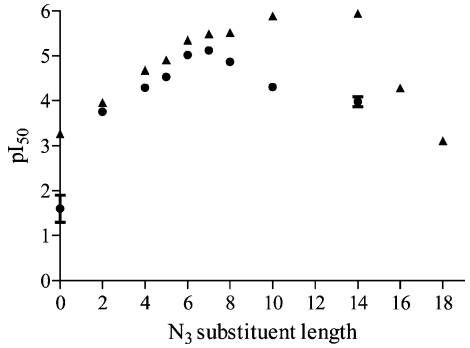
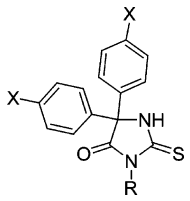


Figure 2. Influence of the N_3 substituent length of the 5,5′-diphenylimidazolidine-2,4-dione (●) and 5,5′-diphenyl-2-thioxoimidazolidin-4-one (▲) derivatives on the FAAH inhibition (pI_{50} ± SEM).

of the alkyl chain length on the enzyme activity is illustrated in Figure 2. The inhibitory potency is the highest for derivatives bearing alkyl chains of six (**13**) or seven (**14**) carbon atoms. Shorter or longer alkyl substituents resulted in loss of activity. For instance, on one hand, 5,5′-diphenylimidazolidine-2,4-dione (**7**), better known as phenytoin, which is unsubstituted in position 3, was unable to inhibit FAAH activity. On the other hand, 5,5′-diphenyl-3-tetradecylimidazolidine-2,4-dione (**24**), which has a rather long substituent, showed a reduced activity (pI_{50} = 3.98 ± 0.11) compared to **13** or **14**.

Considering the 3-octyl derivatives (**17**−**22**), substitution in para position of the phenyl ring by chlorine or bromine atoms

Table 2. 3-Substituted 5,5′-Diphenyl-2-thioxoimidazolidin-4-one Derivatives (**29**−**50**, **54**)[a]



compd	X	R	pI_{50}	% of displacement hCB$_1$ receptor	% of displacement hCB$_2$ receptor
29	H	H	3.27 ± 0.03	<15	<10
30	H	C_2H_5	3.96 ± 0.05	40.0 ± 5.03	<10[b]
31	H	*n*-C_4H_9	4.68 ± 0.03	42.8 ± 4.8[b]	<10[b]
32	Cl	*n*-C_4H_9	4.34 ± 0.09	72.7 ± 4.6	<20
33	Br	*n*-C_4H_9	4.09 ± 0.18	73.8 ± 5.5[b]	<20[b]
34	H	*i*-C_4H_9	3.85 ± 0.09	34.6 ± 3.4[b]	<10[b]
35	H	$(CH_2)_3OCH_3$	3.43 ± 0.07	29.4 ± 3.8[b]	<20[b]
36	H	$(CH_2)_3OH$	<2	20.8 ± 5.3[b]	<20[b]
37	H	C_5H_{11}	4.91 ± 0.08	27.6 ± 3.4	<20
38	H	C_6H_{13}	5.35 ± 0.02	21.9 ± 2.9	<20
39	H	C_7H_{15}	5.49 ± 0.02	28.8 ± 5.1[b]	<20[b]
40	Cl	C_7H_{15}	3.75 ± 0.08	67.8 ± 4.5[b]	<20[b]
41	Br	C_7H_{15}	3.62 ± 0.05	34.2 ± 5.2[b]	<20[b]
42	H	C_8H_{17}	5.52 ± 0.02	38.8 ± 4.3[b]	<20[b]
43	Cl	C_8H_{17}	3.72 ± 0.12	36.6 ± 5.0[b]	<10[b]
44	Br	C_8H_{17}	3.23 ± 0.11	23.4 ± 3.7[b]	<20[b]
45	H	$C_{10}H_{21}$	5.89 ± 0.03	<10	<20
46	H	$C_{14}H_{29}$	5.94 ± 0.05	<10	<20
47	H	$C_{16}H_{33}$	4.28 ± 0.07	< 10	<10
48	H	$C_{18}H_{37}$	3.11 ± 0.06	< 10	<10
49	H	$C_{18}H_{35}$	4.21 ± 0.05	< 10	<10
50	H	C_6H_{11}	4.19 ± 0.09	38.5 ± 3.8[b]	<20[b]
54	H	C_6H_5	4.53 ± 0.18	32.6 ± 3.5	<20
51	H	$CH_2C_6H_5$	4.91 ± 0.10	44.7 ± 5.3[b]	<20[b]
52	Cl	$CH_2C_6H_5$	4.34 ± 0.04	74.2 ± 2.5[b]	<15[b]
53	H	$(CH_2)_2C_6H_5$	4.61 ± 0.09	29.3 ± 5.5[b]	<20[b]

[a] Inhibition potential toward FAAH (rat brain homogenate, pI_{50} ± SEM) and affinity for the hCB$_1$ and hCB$_2$ cannabinoid receptor expressed in CHO cells (% of displacement of bound [^{3}H]-SR141716A and [^{3}H]-CP-55,940, for the hCB$_1$ and hCB$_2$ cannabinoid receptor, respectively, obtained with 10 μM of competitor, mean ± SEM). [b] From ref 32.

resulted in less potent inhibitors as illustrated by compounds **17** (X = H), **19** (X = Cl), and **20** (X = Br), possessing pI_{50} values of 4.87 ± 0.03, 3.62 ± 0.05, and 3.30 ± 0.04, respectively. The methyl (**21**) and methoxy (**22**) moieties were also responsible for a decreased potency with pI_{50} values of 3.79 ± 0.04 and 3.52 ± 0.10, respectively. Interestingly, the fluoro derivative **18** retained the activity of the unsubstituted compound (**17**) (pI_{50} = 4.89 ± 0.05), suggesting that the drop of potency observed with other halogens is more likely due to a steric effect rather than to an electronic one.

When the substituent in position 3 is a phenyl or an alkylphenyl, the highest inhibitory activity was obtained with the benzyl substituent (**26**, pI_{50} = 4.73 ± 0.05) followed by the ethylphenyl (**28**, pI_{50} = 4.30 ± 0.07) and the phenyl (**55**, pI_{50} = 4.13 ± 0.05) substituents.

Similar structure−activity relationships were partially found for the 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives (Table 2 and Figure 2). As observed for the 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives, the activity increases with the length of the alkyl substituent. Compound **30**, bearing an ethyl moiety, possesses a pI_{50} value of 3.96 ± 0.05, while compounds **31** (*n*-butyl) and **38** (*n*-hexyl) have pI_{50} values of 4.68 ± 0.03 and 5.35 ± 0.02, respectively. A decreased activity is also observed for the thio derivatives substituted on the phenyl rings. This is well illustrated by

compounds **39** (X = H), **40** (X = Cl), and **41** (X = Br), exhibiting pI_{50} value of 5.49 ± 0.02, 3.75 ± 0.08, and 3.62 ± 0.05, respectively. And finally, as for the oxo derivatives, a benzyl substituent (**51**, pI_{50} = 4.91 ± 0.10) is preferred to an ethylphenyl (**53**, pI_{50} = 4.61 ± 0.09) or phenyl (**54**, pI_{50} = 4.53 ± 0.18) substituent. However, with the thio derivatives, the drop in the activity is observed with much longer N_3 substituents in contrast to what is observed with the 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives (Figure 2). Indeed, the highest inhibitory activities were obtained for compounds **45** (pI_{50} = 5.89 ± 0.03) and **46** (pI_{50} = 5.94 ± 0.05), possessing a decyl and tetradecyl substituent, respectively, and the activity is reduced for compounds **47** (pI_{50} = 4.28 ± 0.07) and **48** (pI_{50} = 3.11 ± 0.06), possessing an hexadecyl and octadecyl substituent, respectively. One could argue that this activity enhancement when the substituent length increases is due to an overall lipophilic effect. However, this is overruled by compounds **31** and **34**, which are both substituted with a butyl alkyl chain and thus have the same lipophilicity. Compound **31**, substituted with a *n*-butyl chain, possesses a pI_{50} value of 4.68 ± 0.03, whereas compound **34**, substituted with an isobutyl chain, has a pI_{50} value of 3.85 ± 0.09. Interestingly enough, a different evolution of the inhibitory potencies is observed for the oxo and thio derivatives when the alkyl chain length is increased (Figure 2). Previous studies revealed that the differences observed in the electrostatic (molecular electrostatic potential) and lipophilic properties (CLOGP) of the oxo and thio derivatives are so small, that it could hardly explain the differences observed with the in vitro data.[32] More in-depth investigations, such as docking studies, could possibly answer this question.

It is known that unsaturations in the side chain of FAAH inhibitors bearing a fatty acid chain can result in an increased inhibitory activity; therefore, an unsaturation was introduced in the alkyl side chain of derivative **48**, resulting in a compound bearing a octadec-9-enyl substituent (**49**). As expected, the unsaturated side chain induced an increase of the activity (**49**, pI_{50} = 4.21 ± 0.05) compared to the octadecyl substituent (**48**, pI_{50} = 3.11 ± 0.06).

Another interesting point is the drop of activity observed for more polar substituents such as methoxypropyl (**35**, pI_{50} = 3.43 ± 0.07) and, even more dramatically, with hydroxypropyl (**36**, pI_{50} < 2). The activities of these two compounds should be compared with the activity of 3-butyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (**31**), which bears a substituent of similar length (pI_{50} = 4.68 ± 0.03).

Next the structre-activity relationships of changing the substitution pattern around the imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one rings was evaluated. Compounds possessing only one phenyl ring in position 5 (**58**−**65**) or even no phenyl at all (**69**−**73**) were synthesized and their activity toward FAAH hydrolytic activity was evaluated (Tables 3 and 4).

Three compounds substituted with a phenyl in position 5 and with a phenyl ring or a heptyl chain in position 3 were obtained (**58**−**60**, Table 3). The activity of 3,5-diphenylimidazolidine-2,4-dione (**58**, pI_{50} = 3.34 ± 0.04) is significantly lower than the activity of the corresponding 5,5′-diphenyl derivative (**55**, pI_{50} = 4.13 ± 0.05). In a similar way, the inhibitory activity of 3,5-diphenyl-2-thioxoimidazolidin-4-one (**59**, pI_{50} = 3.79 ± 0.04) is lower than the activity of the 5,5′-diphenyl-2-thioxo derivative (**54**, pI_{50} = 4.53 ± 0.18). The same trend was observed when the substituent in position 3 is an alkyl chain, as in the case of 3-heptyl-5-phenyl-2-thioxoimidazolidin-4-one (**60**, pI_{50} = 4.29 ± 0.05) and 3-heptyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (**39**, pI_{50} = 5.49 ± 0.02).

Three 1,5-diphenyl derivatives were also synthesized (**63**−**65**, Table 3). 1,5-Diphenylimidazolidine-2,4-dione (**63**) and 1,5-diphenyl-2-thioxoimidazolidin-4-one (**64**) exhibited very low pI_{50} values (<2 and 3.78 ± 0.02, respectively). In addition, 1,5-diphenyl-3-heptylimidazolidine-2,4-dione (**65**) showed a pI_{50} value of 2.40 ± 0.07, which is much lower than the value obtained for 3-heptyl-5,5′-diphenylimidazolidine-2,4-dione (**14**, pI_{50} = 5.12 ± 0.03).

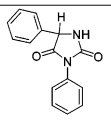
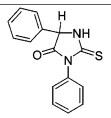
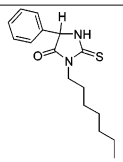
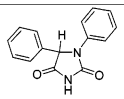
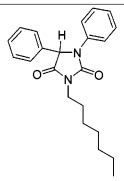
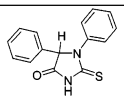
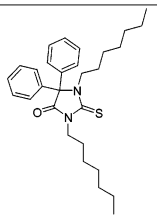
As illustrated above, when derivatives differing in the phenyl rings positions, but bearing the same substituent in position 3, are compared, it appears that in the vast majority of the cases the preferred substitution for FAAH inhibition is the 5,5′-diphenyl one. To confirm the essential role of the phenyl rings in position 5 of the heterocycle, five derivatives (**69**−**73**) without phenyl rings in that position were synthesized (Table 4). None of them displayed a significant inhibition of FAAH hydrolytic activity. For instance, 3-heptylimidazolidine-2,4-dione (**70**) exhibited a pI_{50} of 2.73 ± 0.04 compared to the 5.12 ± 0.03 obtained for compound **14** possessing two phenyls in position 5.

Mode of Inhibition. The mode of inhibition of FAAH activity by these derivatives was investigated using 3-octyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (**42**) as representative compound. Michaelis−Menten curves were constructed, in the presence (12.6 μM) and in the absence of the inhibitor, using increasing concentrations of anandamide (0−70 μM) (Supporting Information). On one hand, the V_{max} values obtained in the presence and absence of the inhibitor (6511 and 6346 pmol/min/mg protein, respectively) are of similar magnitude. On the other hand, K_m values are significantly increased in the presence of the inhibitor (9.6 and 19.9 μM, respectively). These results, representative of two experiments performed in triplicate, illustrate a competitive mode of inhibition, as V_{max} values remain constant while the K_m value is increased in the presence of the inhibitor. Although potent competitive inhibitors of FAAH have already been described (e.g. OL135, or the 2-keto-5-(2-pyridyl)-1,3,4-oxadiazoles), competitive inhibition is not the most common mode of inhibition described so far for FAAH inhibitors.[25] Competitive inhibition of FAAH by 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives could represent an advantage compared to more potent, but irreversible, inhibitors of the enzyme. Indeed, due to their mechanism of action, i.e. a reactive electrophilic center, the irreversible FAAH inhibitors are more likely to interact with other serine hydrolase compared to compounds lacking this kind of electrophilic moiety.

It has been established that the optimum pH value for FAAH activity is 9.[11] Therefore, we assayed two imidazolidine-2,4-diones (**13**, **14**) and two 2-thioxoimidazolidin-4-ones (**45**, **46**) at pH 9. The results obtained and summarized in Table 5 suggest that the FAAH inhibition by the imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives is not influenced by the pH of the medium.

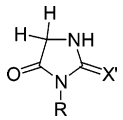
The question whether the 2-thioxoimidazolidin-4-one and imidazolidine-2,4-dione derivatives inhibit FAAH hydrolytic activity by being hydrolyzed instead of anandamide (competing substrates) was also investigated. Preincubations (10 and 20 min) of 3-octyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (**42**) in the presence of brain homogenate prior to anandamide incubation (10 min) were performed. The pI_{50} values obtained were 5.55 ± 0.02 and 5.53 ± 0.03 for 10 and 20 min of preincubation, respectively, and are similar to the value obtained when the homogenate was preincubated alone prior to anandamide

Table 3. Substituted Imidazolidine-2,4-dione and 2-Thioxoimidazolidin-4-one Derivatives (**58**−**60**, **63**−**66**)[a]

Compd.	Formula	pI_{50}	% of displacement - hCB_1 receptor -	% of displacement - hCB_2 receptor -
58		3.34 ± 0.04	<20	<20
59		3.79 ± 0.04	<20	<20
60		4.29 ± 0.05	<20	<20
63		<2	<20	<20
65		2.40 ± 0.07	45.6 ± 5.5	<20
64		3.78 ± 0.02	<20	<20
66		2.07 ± 0.03	<20	<20

[a] Inhibition potential toward FAAH (rat brain homogenate, pI_{50} ± SEM) and affinity for the hCB_1 and hCB_2 cannabinoid receptor expressed in CHO cells (% of displacement of bound [^{3}H]-SR141716A and [^{3}H]-CP-55,940, for the hCB_1 and hCB_2 cannabinoid receptor, respectively, obtained with 10 μM of competitor, mean ± SEM). Compounds **58**−**60**, **63**−**65** were tested as racemic mixtures.

Table 4. Substituted 5,5′-Diphenylimidazolidine-2,4-dione and 5,5′-Diphenyl-2-thioxoimidazolidin-4-one Derivatives (**69**−**73**)[a]



compd	X	R	pI_{50}	% of displacement hCB_1 receptor	% of displacement hCB_2 receptor
69	O	C_4H_9	<10% @ 100 μM	<20	<20
70	O	C_7H_{15}	2.73 ± 0.04	<20	<20
71	O	C_6H_5	23% @ 100 μM	<20	<20
72	S	C_4H_9	<10% @ 100 μM	<20	<20
73	S	C_6H_5	25% @ 100 μM	<20	<20

[a] Inhibition potential toward FAAH (rat brain homogenate) and affinity for the hCB1 and hCB_2 cannabinoid receptor expressed in CHO cells (% of displacement of bound [^{3}H]-SR141716A and [^{3}H]-CP-55,940, for the hCB_1 and hCB_2 cannabinoid receptor, respectively, obtained with 10 μM of competitor, mean ± SEM).

adjunction ($pI_{50} = 5.52 \pm 0.03$). In addition to the preincubation assays, we have incubated the membranes with compound **42**,

Table 5. Influence of the pH on the FAAH Inhibition by Imidazolidine-2,4-dione (**13**, **14**) and 2-Thioxoimidazolidin-4-one (**45**, **46**) Derivatives

compd	X	R	FAAH inhibition pH 7.6	FAAH inhibition pH 9.0
13	O	C_6H_{13}	5.02 ± 0.02	5.00 ± 0.02
14	O	C_7H_{15}	5.12 ± 0.03	5.08 ± 0.05
45	S	$C_{10}H_{21}$	5.89 ± 0.03	5.87 ± 0.04
46	S	$C_{14}H_{29}$	5.94 ± 0.05	6.05 ± 0.04

for 0, 10, or 40 min, and analyzed by HPLC the residue obtained after lyophylization. This procedure allowed an easy and rapid analysis of the entire incubation medium. A phosphate buffer, instead of the usual TRIS−HCl/EDTA buffer, was used in this assay due to UV absorption of TRIS buffers.[37] The pI_{50} value obtained for **42** using the phosphate buffer was similar to the value obtained with the TRIS−HCl/EDTA buffer. The time of retention (3.35 min) and the peak area of compound **42** were found similar, whatever the incubation time. Indeed, no significant differences were found between the peak area values obtained after 0, 10, and 40 min of incubation (one-way ANOVA analysis followed by a Tuckey post-test; data not

shown). Moreover, no significant additional peak was detected after 10 or 40 min of incubation, compared to those detected without incubation. Taken together, the results obtained in these two assays suggest that the 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives do not act as competing substrates of the enzyme.

FAAH Inhibition versus the Cannabinoid Receptor Recognition. 3-Substituted 5,5′-diphenylimidazolidine-2,4-dione and 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one derivatives were previously described as CB_1 cannabinoid receptor selective ligands.[30−32] Therefore, all the new derivatives were screened at 10 μM for competitive binding to the hCB_1 and hCB_2 cannabinoid receptors using membranes of Chinese hamster ovarian (CHO) cells selectively expressing either the hCB_1 or the hCB_2 cannabinoid receptors. None of the compounds displayed significant binding to the hCB_2 cannabinoid receptors, as they displaced less than 20% of the radioligand ([^{3}H]-CP-55,940) bound to the receptor (Tables 1−4). The competitive binding to the hCB_1 cannabinoid receptor, expressed as the displacement percentages of [^{3}H]-SR141716A from its binding site, are listed in Tables 1−4. We have shown previously that the hCB_1 cannabinoid receptor affinity of these derivatives is enhanced by para substitution of the phenyl rings by halogens and by substituting nitrogen 3 with a short alkyl chain (e.g. butyl).[31,32] In the present study, substitution of the phenyls afforded less active inhibitors, and long alkyl chains (from 7 to 14 carbons for the 2-thioxoimidazolidin-4-ones) produced higher FAAH inhibitor activity than the short alkyl chains. This is confirmed, for instance, by compounds **45** and **46**, which displayed a high inhibition of FAAH activity while having no affinity for the hCB_1 cannabinoid receptor. The structure−activity relationships found here for FAAH inhibition, i.e. unsubstituted phenyls and long alkyl chain, are the opposite of the structure−activity relationships found at the CB_1 cannabinoid receptor, i.e. substituted phenyls and short alkyl chain. It is therefore possible to favor either the affinity for the CB_1 cannabinoid receptor or the FAAH inhibition potential of the imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one by modulating the nature and position of the substituents around the central scaffold.

In conclusion, we have described for the first time the inhibitor potential toward FAAH activity of imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one derivatives. The latter are more potent inhibitors than the corresponding oxo derivative. They act as competitive inhibitors of FAAH activity without being hydrolyzed by the enzyme. Among them, 5,5′-diphenyl-3-tetradecyl-2-thioxoimidazolidin-4-one (**46**) showed the highest activity ($pI_{50} = 5.94$) for the enzyme, while displaying no affinity for the cannabinoid receptors. Actually, unsubstituted phenyls combined with N_3 long alkyl chains enhance the inhibitory activity for the enzyme, while halogen substitution on the phenyls along with N_3 short alkyl chains enhance CB_1 cannabinoid receptor affinity. Finally, 3-substituted 5,5′-diphenyl-2-thioxoimidazolidin-4-one could constitute an interesting template for the development of more potent reversible and competitive FAAH inhibitors.

Experimental Section

General Procedures. All reagents were purchased from commercial sources (Sigma-Aldrich or Acros) and were used without further purification. Solvents were of analytical grade. [^{3}H]-AEA (60 Ci/mmol) was purchased from American Radiolabeled Chemical (St Louis, MO), [^{3}H]-SR141716A (52 Ci/mol) from Amersham (Roosendaal, The Netherlands), and [^{3}H]-CP-55,940 (101 Ci/mol) from NEN Life Science (Zaventem, Belgium). HU-210 was purchased from Tocris (Bristol, UK).

The microwave oven used was a commercial household microwave oven (frequency 2450 MHz). Melting points (mp) were determined in open capillaries using a Electrothermal 9100 apparatus and are reported uncorrected. Infrared (IR) spectra of compounds, dispersed in KBr, were recorded using a Perkin-Elmer FT-IR 286 spectrometer, and values are reported as ν in cm^{-1} (see Supporting Information). Nuclear magnetic resonance (^{1}H NMR, ^{13}C NMR) spectra were recorded on a Bruker AM-300 spectrometer at room temperature and analyzed using the WIN NMR software package. Chemical shifts (δ) are reported relative to the tetramethylsilane peak set at 0.00 ppm. In the case of multiplets, the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants are expressed in hertz. Mass spectra were recorded on a Finnigan MAT 44S, with an ionization voltage of 70 eV. Elemental analyses of original compounds were performed on a Carlo Erba EA 1108 Analyzer (Carlo Erba, Milano, Italy) and are within ±0.4% of the theoretical values.

Synthesis. General Procedure for the Synthesis of the 3-Substituted 5,5′-Diphenylimidazolidine-2,4-diones. These compounds were obtained following a microwave-enhanced method previously described.[33] Briefly, to a solution of benzil (9.9 mmol) and substituted urea (19.9 mmol) in 25 mL of DMSO was added 15 mL of a 1.2 M KOH solution with stirring. Following an initial 90-s microwave irradiation (750 W), the mixture was stirred for 5 min. Eight 30-s microwave pulses were applied at the time points of 6, 9, 12, 15, 18, 21, 24, and 30 min. The mixture was stirred between pulses. At the end of the sequence, the mixture was poured onto crushed ice and the precipitate filtered off. The residue was subsequently crystallized.

Compounds **9-13**, **15-18**, **20**, **25**, **26**, and **28** were previously described by us.[30,33,34]

3-Ethyl-5,5′-diphenylimidazolidine-2,4-dione (8). Yield: 60%. Mp: 137.9−139.2 °C. MS (EI): 280 [M]$^+$. ^{13}C NMR (DMSO-d_6): δ 13.37 (CH_3), 33.29 (CH_2), 67.14 (C), 126.72, 128.27, 128.72, 139.79 (C_{arom} and CH_{arom}), 155.31 (C═O), 173.11 (C═O).

3-Heptyl-5,5′-diphenylimidazolidine-2,4-dione (14). Yield: 55%. Mp: 82.3−83.4 °C. MS (EI): 350 [M]$^+$. ^{13}C NMR (DMSO-d_6): δ 13.78 (CH_3), 21.74 (CH_2), 25.72 (CH_2), 27.19 (CH_2), 27.90 (CH_2), 30.94 (CH_2), 37.86 (CH_2), 68.90 (C), 126.46, 128.02, 128.44, 139.60 (C_{arom} and CH_{arom}), 155.31 (C═O), 173.09 (C═O). Anal. Calcd for $C_{22}H_{26}N_2O_2$: C, H, N.

5,5′-Bis(4-chlorophenyl)-3-octylimidazolidine-2,4-dione (19). Yield: 47%. Mp: 129.4−130.3 °C. MS (EI): 432 [M]$^+$. ^{13}C NMR (DMSO-d_6): δ 13.78 (CH_3), 21.93 (CH_2), 25.81 (CH_2), 27.11 (CH_2), 28.27 (CH_2), 28.40 (CH_2), 38.40 (CH_2), 38.11 (CH_2), 68.06 (C), 128.42, 128.62, 133.15, 138.26 (C_{arom} and CH_{arom}), 155.15 (C═O), 172.61 (C═O). Anal. Calcd for $C_{23}H_{26}Cl_2N_2O_2$: C, H, N.

5,5′-Bis(4-methylphenyl)-3-octylimidazolidine-2,4-dione (21). Yield: 45%. Mp: 107.8−108.9 °C. MS (EI): 393 [M]$^+$. ^{13}C NMR (DMSO-d_6): δ 14.07 (CH_3), 21.06 (2xCH_3), 22.64 (CH_2), 24.54 (CH_2), 26.62 (CH_2), 28.01 (CH_2), 29.11 (CH_2), 31.73 (CH_2), 39.08 (CH_2), 68.54 (C), 126.74, 129.43, 136.51, 138.36 (C_{arom} and CH_{arom}), 155.45 (C═O), 172.83 (C═O). Anal. Calcd for $C_{25}H_{32}N_2O_2$: C, H, N.

5,5′-Bis(4-methoxyphenyl)-3-octylimidazolidine-2,4-dione (22). Yield: 48%. Mp: 87.8−88.9 °C. MS (EI): 424 [M]$^+$. ^{13}C NMR (DMSO-d_6): δ 14.01 (CH_3), 22.17 (CH_2), 25.98 (CH_2), 27.47 (CH_2), 28.44 (CH_2), 28.57 (CH_2), 31.16 (CH_2), 38.02 (CH_2), 55.29 (2xCH_3), 68.29 (C), 113.91, 128.01, 132.03 (C_{arom} and CH_{arom}), 155.51 (C═O), 159.07 (C_{arom}), 173.89 (C═O). Anal. Calcd for $C_{25}H_{32}N_2O_4$: C, H, N.

3-Decyl-5,5′-diphenylimidazolidine-2,4-dione (23). Yield: 48%. Mp: 72.4−73.8 °C. MS (EI): 392 [M]$^+$. ^{13}C NMR ($CDCl_3$): δ 13.85 (CH_3), 21.93 (CH_2), 25.75 (CH_2), 27.17 (CH_2), 28.27 (CH_2), 28.53 (CH_2), 28.73 (CH_2), 31.12 (CH_2), 37.85 (CH_2), 68.91 (C), 126.49, 127.98, 128.53, 139.75 (C and CH arom.), 155.23 (C═O), 173.17 (C═O). Anal. Calcd for $C_{25}H_{32}N_2O_2$: C, H, N.

5,5′-Diphenyl-3-tetradecylimidazolidine-2,4-dione (24). Yield: 30%. Mp: 69.1−70.5 °C. MS (EI): 449 $[M + H]^+$. ^{13}C NMR ($CDCl_3$): δ 14.11 (CH_3), 22.65 (CH_2), 26.59 (CH_2), 27.06 (CH_2), 27.95 (CH_2), 28.53 (CH_2), 29.05 (CH_2), 29.16 (CH_2), 29.32 (CH_2), 29.45 (CH_2), 29.64 (CH_2), 30.03 (CH_2), 31.90 (CH_2), 39.09 (CH_2), 70.01 (C), 126.82, 128.44, 128.76, 139.31 (C_{arom} and CH_{arom}), 156.84 (C═O), 173.34 (C═O). Anal. Calcd for $C_{29}H_{40}N_2O_2$: C, H, N.

3-Benzyl-5,5′-bis(4-chlorophenyl)imidazolidine-2,4-dione (27). Yield: 52%. Mp: 156.8−157.6 °C. MS (EI): 412 $[M + H]^+$. ^{13}C NMR (DMSO-d_6) δ 41.03 (CH_2), 68.56 (C), 126.39, 127.63, 127.88, 128.47, 128.86, 129.70, 129.89, 130.15, 133.51, 133.64, 136.62, 138.30 (C_{arom} and CH_{arom}), 155.25 (C═O), 172.66 (C═O). Anal. Calcd for $C_{22}H_{16}Cl_2N_2O_2$: C, H, N.

General Procedure for the Synthesis of the 3-Substituted 5,5′-Diphenyl-2-thioxoimidazolidin-4-ones. These compounds were obtained similarly to the 3-substituted 5,5′-diphenylimidazolidine-2,4-dione derivatives starting from the corresponding thiourea. Syntheses of compounds **30**, **31**, **33**−**36**, **39**−**44**, **50**−**53**,[32] and **54**[34] were described elsewhere. For compounds **46**−**49**, the residue obtained following the reaction was extracted with $CHCl_3$, and the organic layer was subsequently washed with HCl, NaOH, and brine. The resulting organic phase was dried over $MgSO_4$ and evaporated to dryness.

5,5′-Bis(4-chlorophenyl)-3-butyl-2-thioxoimidazolidin-4-one (32). Yield: 51%. Mp: 153.8−154.6 °C. MS (EI): 393 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 13.63 (CH_3), 19.38 (CH_2), 29.35 (CH_2), 40.61 (CH_2), 70.24 (C), 128.59, 129.1, 133.70, 136.87 (C_{arom} and CH_{arom}), 173.11 (C═O), 181.52 (C═S). Anal. Calcd for $C_{19}H_{18}Cl_2N_2OS$: C, H, N.

3-Pentyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (37). Yield: 71%. Mp: 108.3−109.1 °C. MS (EI): 338 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 13.56 (CH_3), 21.45 (CH_2), 26.56 (CH_2), 27.92 (CH_2), 41.31 (CH_2), 70.01 (C), 126.46, 128.34, 128.60, 138.04 (C_{arom} and CH_{arom}), 173.37 (C═O), 181.13 (C═S). Anal. Calcd for $C_{20}H_{22}N_2OS$: C, H, N.

3-Hexyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (38). Yield: 69%. Mp: 108.6−109.7 °C. MS (EI): 352 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 13.56 (CH_3), 21.71 (CH_2), 25.40 (CH_2), 26.82 (CH_2), 30.51 (CH_2), 40.51 (CH_2), 70.01 (C), 126.01, 126.46, 128.34, 138.04 (C_{arom} and CH_{arom}), 173.37 (C═O), 181.13 (C═S). Anal. Calcd for $C_{21}H_{24}N_2OS$: C, H, N.

3-Decyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (45). Yield: 15%. Mp: 54.4−55.5 °C. MS (EI): 408 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 14.34 (CH_3), 22.49 (CH_2), 26.31 (CH_2), 27.47 (CH_2), 28.89 (CH_2), 29.09 (CH_2), 29.22 (CH_2), 29.57 (CH_2), 31.68 (CH_2), 40.85 (CH_2), 71.53 (C), 126.98, 128.86, 129.12, 138.69 (C_{arom} and CH_{arom}), 173.88 (C═O), 181.71 (C═S). Anal. Calcd for $C_{25}H_{32}N_2OS$: C, H, N.

5,5′-Diphenyl-3-tetradecyl-2-thioxoimidazolidin-4-one (46). Yield: 17%. Mp: 46.5−47.7 °C. MS (EI): 464 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 13.75 (CH_3), 21.90 (CH_2), 25.72 (CH_2), 26.82 (CH_2), 27.65 (CH_2), 28.24 (CH_2), 28.63 (CH_2), 28.85 (CH_2), 28.98 (CH_2), 29.22 (CH_2), 29.41 (CH_2), 31.16 (CH_2), 31.27 (CH_2), 39.09 (CH_2), 70.95 (C), 126.20, 127.50, 128.60, 138.04 (C_{arom} and CH_{arom}), 173.37 (C═O), 181.13 (C═S). Anal. Calcd for $C_{29}H_{40}N_2OS$: C, H, N.

3-Hexadecyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (47). Yield: 19%. Mp: 63.7−64.6 °C. MS (EI): 492 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 14.50 (CH_3), 23.11 (CH_2), 27.06 (CH_2), 27.65 (CH_2), 28.02 (CH_2), 28.24 (CH_2), 28.63 (CH_2), 28.85 (CH_2), 28.98 (CH_2), 29.19 (CH_2), 29.51 (CH_2), 29.77 (CH_2), 29.90 (CH_2), 30.09 (CH_2), 32.36 (CH_2), 41.13 (CH_2), 72.40 (C), 127.34, 127.79, 129.34, 138.40 (C_{arom} and CH_{arom}), 173.92 (C═O), 183.04 (C═S). Anal. Calcd for $C_{31}H_{44}N_2OS$: C, H, N.

3-Octadecyl-5,5′-diphenyl-2-thioxoimidazolidin-4-one (48). Yield: 16%. Mp: 64.1−65.0 °C. MS (EI): 520 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 14.04 (CH_3), 22.65 (CH_2), 26.60 (CH_2), 26.92 (CH_2), 27.33 (CH_2), 27.57 (CH_2), 27.76 (CH_2), 27.91 (CH_2), 28.35 (CH_2), 28.57 (CH_2), 28.73 (CH_2), 29.06 (CH_2), 29.64 (CH_2), 31.90 (CH_2), 41.61 (CH_2), 72.09 (C), 126.30, 128.11, 128.69, 137.94 (C_{arom} and CH_{arom}), 173.47 (C═O), 182.53 (C═S). Anal. Calcd for $C_{33}H_{48}N_2OS$: C, H, N.

3-((*Z*)-Octadec-9-enyl)-5,5′-diphenyl-2-thioxoimidazolidin-4-one (49). Yield: 21%. Oily compound. MS (EI): 518 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 13.44 (CH_3), 23.04 (CH_2), 26.46 (CH_2), 26.99 (CH_2), 27.34 (CH_2), 27.56 (CH_2), 27.96 (CH_2), 28.65 (CH_2), 29.19 (CH_2), 30.03 (CH_2), 32.29 (CH_2), 32.93 (CH_2), 41.99 (CH_2), 72.34 (C), 127.27, 127.66, 129.08 (C_{arom} and CH_{arom}), 129.28 (CH), 138.40 (C_{arom}), 173.98 (C═O), 181.49 (C═S). Anal. Calcd for $C_{33}H_{46}N_2OS$: C, H, N.

3,5,5′-Triphenylimidazolidine-2,4-dione (55). 3,5,5′-Triphenyl-2-thioxoimidazolidin-4-one (**54**) was dissolved in a mixture of DMF (10 mL) and acetic acid (1 mL). After addition of hydrogen peroxide (30%, 2.5 mL), the solution was stirred for 24 h at room temperature and poured onto ice. The precipitate was collected, dried, and crystallized from ethanol. Yield: 80%. 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_{arom} and CH_{arom}), 154.28 (C═O), 172.46 (C═O).

3,5-Diphenylimidazolidine-2,4-dione (58). Phenylglycine methyl ester (**56**; 6.6 mmol) and phenyl isocyanate (**57**, X′ = O; 6.6 mmol) were vigorously stirred for 2 h at room temperature in pyridine (30 mL). The resulting mixture was evaporated to dryness under reduced pressure. Aqueous HCl (30 mL, 2 N) was added to the residue and the mixture refluxed for 4 h. A precipitate appeared upon cooling and was recrystallized from hot water. Yield: 25%. Mp: 192.3−192.8 °C. MS (DCI): 253 $[M + H]^+$. ^{13}C NMR (DMSO-d_6): δ 59.82 (CH), 126.64, 126.95, 127.69, 128.34, 128.66, 135.58, (C_{arom} and CH_{arom}), 155.57 (C═O), 171.49 (C═O).

3,5-Diphenyl-2-thioxoimidazolidin-4-one (59). The compound was synthesized as **58** using phenyl isothiocyanate (**57**, X′ = S) instead of phenyl isocyanate. Yield: 30%. Mp: 232.1−233.6 °C. MS (DCI): 269 $[M + H]^+$. ^{13}C NMR (DMSO-d_6): δ 62.80 (CH), 127.34, 128.85, 128.99, 129.13, 129.66, 133.58, (C_{arom} and CH_{arom}), 172.97 (C═O), 182.91 (C═S).

3-Heptyl-5-phenyl-2-thioxoimidazolidin-4-one (60). The compound was synthesized as **58** using heptyl isothiocyanate (**57**, X′ = S) instead of phenyl isocyanate. Yield: 25%. Mp: 98.6−99.2 °C. MS (EI): 290 $[M]^+$. ^{13}C NMR (DMSO-d_6) δ 14.44 (CH_3), 22.91 (CH_2), 27.05 (CH_2), 27.96 (CH_2), 29.19 (CH_2), 32.10 (CH_2), 42.06 (CH_2), 63.02 (CH), 127.01, 127.14, 129.79, 133.68 (C_{arom} and CH_{arom}), 172.75 (C═O), 184.92 (C═S). Anal. Calcd for $C_{16}H_{22}N_2OS$: C, H, N.

General Procedure for the Synthesis of the 1,5-Diphenyl Derivatives. These compounds were synthesized following a method adapted from Joshi et al.[35] Briefly, phenylglyoxal hydrate (**61**; 4.5 mmol) and phenylurea (**62**, X′ = O) or phenylthiourea (**62**, X′ = S) (4.5 mmol) were refluxed for 4 h in 15 mL of glacial acetic acid in the presence of 0.5 mL of hydrochloric acid. After cooling, the mixture was poured in water and the resulting precipitate filtered off. The residue was subsequently crystallized from ethanol.

1,5-Diphenylimidazolidine-2,4-dione (63). Yield: 68%. Mp: 197.6−198.9 °C. MS (EI): 252 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 64.73 (CH), 121.15, 124.45, 127.49, 128.85, 128.98, 129.17, 134.35, 136.81 (C_{arom} and CH_{arom}), 155.12 (C═O), 171.81 (C═O).

1,5-Diphenyl-2-thioxoimidazolidin-4-one (64). Yield: 70%. Mp: 234.1−235.6 °C. MS (EI): 268 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 63.19 (CH), 126.14, 127.56, 128.21, 128.34, 129.24, 129.44, 133.77, 134.87 (C_{arom} and CH_{arom}), 173.11 (C═O), 183.20 (C═S).

3-Heptyl-1,5-diphenylimidazolidine-2,4-dione (65). This compound was obtained by alkylation of **63**. Briefly, **63** (2.5 mmol) and K_2CO_3 (5 mmol) were stirred in DMF (4 mL) for 30 min prior to addition of chloroheptane (3.25 mmol). The resulting mixture was stirred for 24 h and poured in water. The solution was extracted with $CHCl_3$, and the organic layer was washed with HCl, NaOH, and brine. The resulting organic phase was dried over $MgSO_4$ and evaporated to dryness. Yield: 35%. Mp: 66.5−67.8 °C. MS (EI): 350 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 13.62 (CH_3), 21.71 (CH_2), 25.72 (CH_2), 27.08 (CH_2), 27.86 (CH_2), 30.84 (CH_2), 62.92 (CH),

120.70, 124.13, 126.98, 128.40, 128.53, 128.73, 133.64, 136.17 (C_{arom} and CH_{arom}), 155.15 (C═O), 170.07 (C═O).

1,3-Diheptyl-5,5′-diphenylimidazolidine-2,4-dione (66). Compound **66** was obtained by alkylation of 5,5′-diphenylimidazolidine-2,4-dione (**7**). The 5,5′-diphenylimidazolidine-2,4-dione (4 mmol) and K_2CO_3 (12 mmol) were stirred in DMF (40 mL) for 30 min prior to addition of chloroheptane (8 mmol). The resulting mixture was stirred for 24 h and poured in water. The solution was extracted with $CHCl_3$, and the organic layer was washed with HCl, NaOH, and brine. The resulting organic phase was dried over $MgSO_4$ and evaporated to dryness to yield a colorless oil. Yield: 16%. MS (EI): 448 $[M]^+$. ^{13}C NMR ($CDCl_3$): δ 13.75 (CH_3), 13.80 (CH_3), 21.79 (CH_2), 25.75 (CH_2), 26.32 (CH_2), 27.24 (CH_2), 27.29 (CH_2), 27.88 (CH_2), 27.97 (CH_2), 30.76 (CH_2), 31.01 (CH_2), 38.33 (CH_2), 41.26 (CH_2), 73.98 (C), 127.99, 128.69, 137.16, 138.74 (C_{arom} and CH_{arom}), 154.92 (C═O), 173.01 (C═O). Anal. Calcd for $C_{29}H_{40}N_2O_2$: C, H, N.

General Procedure for the Synthesis of the 3-Substituted Derivatives. To the glycine ethyl ester isothiocyanate or isocyanate (7 mmol) dissolved in $CHCl_3$ (25 mL) was added, over a 10 min period, the amine (7 mmol) in 15 mL of $CHCl_3$. After 4 h of stirring, the solution was evaporated to dryness. The resulting residue was refluxed for 3 h in 50 mL of a 1:1 mixture of ethanol and HCl (10 M). After cooling, the solution was evaporated to dryness and the resulting solid crystallized from hexane.

3-Butylimidazolidine-2,4-dione (69). Analytical data are in accordance with those reported by Ryczek.[36] Yield: 74%. Mp: 97.1−98.2 °C. MS (EI): 157 $[M + H]^+$. ^{13}C NMR (DMSO-d_6): δ 13.39 (CH_3), 19.28 (CH_2), 29.56 (CH_2), 37.20 (CH_2), 45.74 (CH_2), 157.70 (C═O), 171.97 (C═O).

3-Heptylimidazolidine-2,4-dione (70). Analytical data are in accordance with those reported by Ryczek.[36]Yield: 77%. Mp: 81.1−81.9 °C. MS (EI): 198 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 14.01 (CH_3), 22.10 (CH_2), 26.17 (CH_2), 27.66 (CH_2), 28.31 (CH_2), 31.22 (CH_2), 37.63 (CH_2), 45.91 (CH_2), 157.77 (C═O), 172.14 (C═O).

3-Phenylimidazolidine-2,4-dione (71). Analytical data are in accordance with those reported by Ryczek.[36] Yield: 67%. Mp: 158.2−159.5 °C. MS (EI): 176 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 46.17 (CH_2), 126.8, 127.76, 128.79, 132.41 (C_{arom} and CH_{arom}), 156.61 (C═O), 171.17 (C═O).

3-Butyl-2-thioxoimidazolidin-4-one (72). Analytical data are in accordance with those reported by Ryczek.[36] Yield: 64%. Mp: 109.8−110.7 °C. MS (EI): 172 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 13.69 (CH_3), 19.51 (CH_2), 29.47 (CH_2), 38.86 (CH_2), 48.44 (CH_2), 172.78 (C═O), 183.59 (C═S).

3-Phenyl-2-thioxoimidazolidin-4-one (73). Analytical data are in accordance with those reported by Ryczek.[36] Yield: 60%. Mp: 258.3−259.7 °C. MS (EI): 192 $[M]^+$. ^{13}C NMR (DMSO-d_6): δ 49.27 (CH_2), 128.66, 128.79, 128.92, 133.64 (C_{arom} and CH_{arom}), 172.26 (C═O), 183.53 (C═S).

Pharmacology. Brain Membrane Preparation. Male Wistar rats (250−300 g) were purchased from IFFA-CREDO (Les Oncins, France). All experiments on animals were approved by the institutional ethics committee, and the housing conditions were as specified by the Belgian Law of November 14, 1993, on the protection of laboratory animals (agreement no. LA 1230315). Following decapitation, brains were carefully dissected on ice. All the manipulations were performed at 0−4 °C. Adult rat brain was homogenized in homogenization buffer (20 mM HEPES, 1 mM $MgCl_2$, pH 7.0) using a potter and subsequently centrifuged for 20 min at 36 000*g*. The pellet was resuspended in homogenization buffer and centrifuged again for 20 min at 36 000*g*. The latter operation was performed twice. The resulting pellet was stored in a conservation buffer (50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.6). The protein content of the preparation was determined,[38] and the membranes aliquots were stored at −80 °C until use.

Fatty Acid Amide Hydrolase Inhibition Assay. The method is adapted from Omeir et al.[39] Membranes, test compounds or DMSO (10 μL), [^{3}H]-AEA (50.000 dpm; 2 μM final concentration), and assay buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1% (w/v) BSA, pH 7.6 or 9) were incubated at 37 °C for 10 min, in 200 μL final volume. Reactions were stopped by rapidly placing the tubes in ice and adding 400 μL of cold chloroform/methanol (1:1 v/v) followed by vigorous mixing. Phases were separated by centrifugation at 850*g*, and aliquots (200 μL) of the upper methanol/buffer phase were counted for radioactivity by liquid scintillation counting (Pharmacia Wallac 1410 β-counter). Blanks contained buffer instead of the homogenate preparations.

HPLC Assay. The HPLC system consisted of a Perkin-Elmer 10 pump, a Perkin-Elmer LC-85B UV detector, and a Perkin-Elmer LCI-100 integrator. The separations were performed using a 250 × 4.6 mm HPLC column from Bio-Rad (RoSil C18 HL 5 μm). The mobile phase was MeOH (analytical grade) delivered at 1 mL/min. UV detection was carried out at 285 nm, which is the λ_{max} for compound **42** under the assay conditions. Membranes, compound **42** or DMSO (10 μL), and assay buffer ($H_2PO_4^-$−HPO_4^{2-}, 0.1% BSA, pH 7.6) were incubated at 37 °C for 0, 10, or 40 min in 200 μL final volume. Reactions were stopped by placing the tubes in a dry ice−2-propanol mixture. The frozen solutions were subsequently lyophilized. Methanol (200 μL) was added to each tube prior to injection of a 20 μL aliquot into the HPLC.

Competition Binding Assay. The assay was performed as previously described.[30] Briefly, the competitive binding experiments were performed using [^{3}H]-SR141716A (1 nM) or [^{3}H]-CP-55,940 (1nM) as radioligands for the hCB_1 and the hCB_2 cannabinoid receptors respectively, at 30 °C in plastic tubes, and 40 μg of membranes per tube resuspended in 0.5 mL (final volume) binding buffer (50 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). The test compounds were present at a 10 μM concentration and the nonspecific binding was determined in the presence of 10 μM HU-210. After 1 h the incubation was stopped, and solutions were rapidly filtered through 0.5% PEI pretreated GF/B glass fiber filters (Whatman, Maidstone, UK) on a M-48T Brandell cell harvester and washed twice with 5 mL ice-cold binding buffer without serum albumin. The radioactivity on the filters was measured in a Pharmacia Wallac 1410 β-counter using 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. Final DMSO concentrations in the assay were less than 0.1%.

Data Analysis. Inhibition curves of FAAH were performed at least three times in duplicate. IC_{50} values were determined by nonlinear regression analysis performed using the GraphPad prism 4.0 program (GraphPad Software, San Diego) and expressed as pI_{50} ($pI_{50} = -\log IC_{50}$). Competitive binding assays on the cannabinoid receptors (CB_1 and CB_2) were performed at least three times in duplicate. Results are expressed as mean ± SEM.

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Supporting Information Available: Characteristic IR peaks, 1H NMR and elemental analysis data, and the Michaelis-Menten. This material is available free of charge via the Internet at http://pubs.acs.org.

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