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Identification and Characterization of Carprofen as a Multitarget Fatty Acid Amide Hydrolase/Cyclooxygenase Inhibitor

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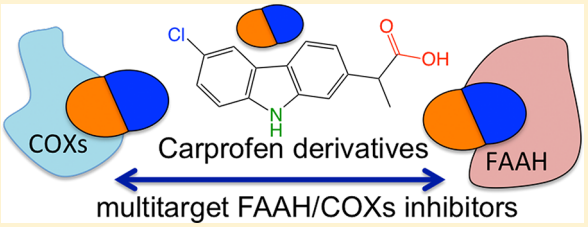
Supporting Information

ABSTRACT: Pain and inflammation are major therapeutic areas for drug discovery. Current drugs for these pathologies have limited efficacy, however, and often cause a number of unwanted side effects. In the present study, we identify the nonsteroidal anti-inflammatory drug carprofen as a multitarget-directed ligand that simultaneously inhibits cyclooxygenase-1 (COX-1), COX-2, and fatty acid amide hydrolase (FAAH). Additionally, we synthesized and tested several derivatives of carprofen, sharing this multitarget activity. This may result in improved analgesic efficacy and reduced side effects (Naidu et al. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 48−56; Fowler, C. J.; et al. *J. Enzyme Inhib. Med. Chem.* **2012**, in press; Sasso et al. *Pharmacol. Res.* **2012**, *65*, 553). The new compounds are among the most potent multitarget FAAH/COX inhibitors reported so far in the literature and thus may represent promising starting points for the discovery of new analgesic and anti-inflammatory drugs.



INTRODUCTION

Pain and inflammation remain areas of substantial unmet patient need.[1−7] Current drugs used to treat these conditions have, however, moderate efficacy and can produce a variety of untoward side effects, such as gastrointestinal bleeding and ulceration, renal dysfunction, nausea, and vomiting. Therefore, the search for novel and more effective analgesics able to overcome these limitations is the subject of intense efforts in both academia and industry.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat acute and chronic pain. NSAIDs produce their beneficial action by inhibiting the two isoforms of the cyclooxygenase (COX) enzyme, COX-1 and COX-2.[8,9] These enzymes convert arachidonic acid into prostaglandins and thromboxane, which are important physiological and pathological effectors. Different tissues express varying levels of COX-1 and COX-2. COX-1 is a constitutive enzyme found in most mammalian cells. COX-2, on the other hand, is an inducible enzyme whose expression can be strongly stimulated by pro-inflammatory stimuli in macrophages and other cells.[10] There are several well-known classes of NSAIDs, which are either nonselective for COX-1 and COX-2 or selective for COX-2.[11] Both classes exert, however, a number of potentially serious side effects.[12] In the gastrointestinal tract, COX-1 inhibition blocks the synthesis of tissue-protecting prostaglandins such as PGE_2, facilitating the development of peptic ulceration and dyspepsia. Selective COX-2 inhibitors have raised major concerns because of increased cardiovascular risk. A notable example is rofecoxib, which was withdrawn from the market in 2004 because of such—still debated—concerns.[13,14]

Fatty acid amide hydrolase (FAAH) has been proposed as a promising target for the discovery of new drugs to treat pain, inflammation, and other pathologies.[15−19] FAAH is an intracellular serine hydrolase responsible for the deactivating hydrolysis of a family of naturally occurring fatty acid ethanolamides, such as its main substrate anandamide, which acts as an endogenous cannabinoid agonist.[20−22] Interestingly, it has been suggested that drugs currently marketed as analgesics may derive some of their efficacy from inhibition of FAAH, which further highlights the potential of this target for drug discovery.[23,24] Several classes of FAAH inhibitors have been discovered during the past decade—including α-ketoheterocycles and carbamate, piperidine and piperazine urea-based molecules—some of which are undergoing preclinical and clinical studies.[25−31]

Several in vivo studies suggest that the simultaneous inhibition of COX and FAAH activities produces superadditive pharmacological effects and lowered toxicity in animal models. Naidu et al. showed that the FAAH inhibitor URB597[32] and the nonselective COX inhibitor diclofenac act synergistically to reduce visceral pain in mice.[33] Similar results were obtained by

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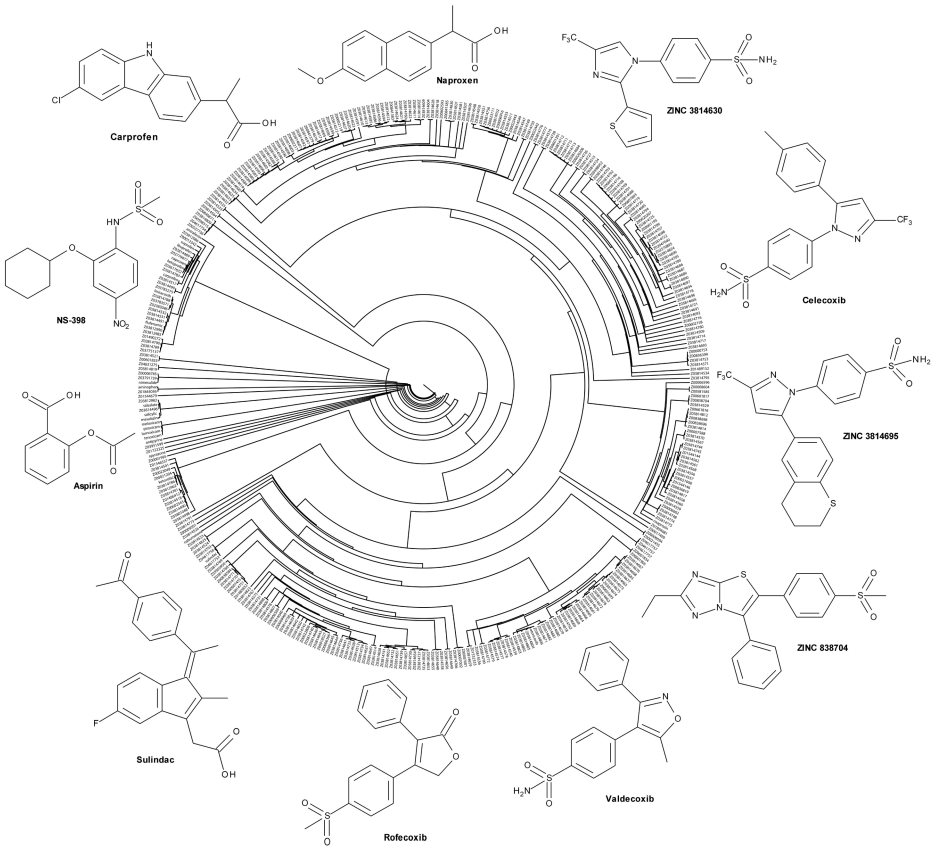

Figure 1. Circular tree based on pairwise Tanimoto distances between Daylight fingerprints of 382 diverse known COX inhibitors. To help in the interpretation, only selected molecules, belonging to different clusters, are depicted in proximity of their positions in the tree to highlight the structural diversity of the set. Carprofen is shown in the upper left corner.

Sasso et al. using the peripherally restricted FAAH inhibitor URB937 and the NSAID indomethacin.[34] Importantly, both studies showed that FAAH blockade lowers the ulcerogenic activity of COX inhibitors.[11] These findings suggest that multitarget-directed ligands[35] able to simultaneously inhibit FAAH and COX activities might offer certain advantages over traditional single-target drugs and/or drug combinations. These include (i) improved efficacy, due to the synergistic interaction between FAAH and COX blockade, (ii) improved safety, due to the lowering of COX-mediated side effects produced by FAAH inhibition, and (iii) reduced uncertainty in clinical development with respect to drug cocktails or multicomponent drugs, due to the avoided risk of drug−drug interactions.[35−38] It is worth remembering that some very successful drugs act via multiple-target mechanisms (e.g., quetiapine, imatinib).

Here, we report on the discovery of new multitarget inhibitors that show improved potency compared to previously reported mixed FAAH/COX compounds.[23,24,39] We used docking calculations to identify putative FAAH/COX inhibitors starting from known COX-targeting drugs. In vitro pharmacological tests identified carprofen (Figure 1) as a multitarget FAAH/COX hit. On the basis of this finding, we designed several carprofen derivatives that showed significant multitarget inhibitory activity, highlighting the potential of the carprofen scaffold as a source for new effective and safe analgesics.

RESULTS

Identification of Carprofen as a Multitarget FAAH/COX Inhibitor. We selected 382 COX inhibitors retrieved from DrugBank[40] and DUD[41] and docked them into the structure of FAAH (see the Experimental Section). Several clinically approved drugs were found among the top-ranking molecules. The entire assembled set was clustered according to pairwise Tanimoto distances, using a description based on the Daylight fingerprints (Figure 1). A clustering threshold of 0.4 resulted in 84 clusters, which highlighted the structural diversity within the set. The top 100 scored molecules were visually inspected. Among them, indomethacin was ranked no. 4, flurbiprofen no. 10, and celecoxib no. 16 (the putative binding mode of these three COX inhibitors at the active site of FAAH is reported in Figure S2, Supporting Information). On the basis of their commercial availability, we purchased 25 compounds for testing (see Figure 2). These molecules were also clustered according to their topological distance and physicochemical similarity, as highlighted in Figure 2. According to the reported tree, a clustering threshold of 0.3 originated 19 clusters, underlining the absence of trivially similar compounds. This unanticipated chemotype richness enabled us to probe FAAH binding thoroughly and maximize the chance to find a multitarget FAAH/COX inhibitor among the 25 selected compounds. The in vitro results identified carprofen (**1**) as the best multitarget FAAH/COX inhibitor in the set of compounds tested. Carprofen inhibited FAAH with a median effective concentration (IC_{50}) of 79 μM, COX-1 with an IC_{50} = 22 μM, and COX-2 with an IC_{50} = 4 μM. As the profile against the three targets turned out to be rather balanced, compound **1** emerged as a promising starting point for multitarget FAAH/COX lead discovery.

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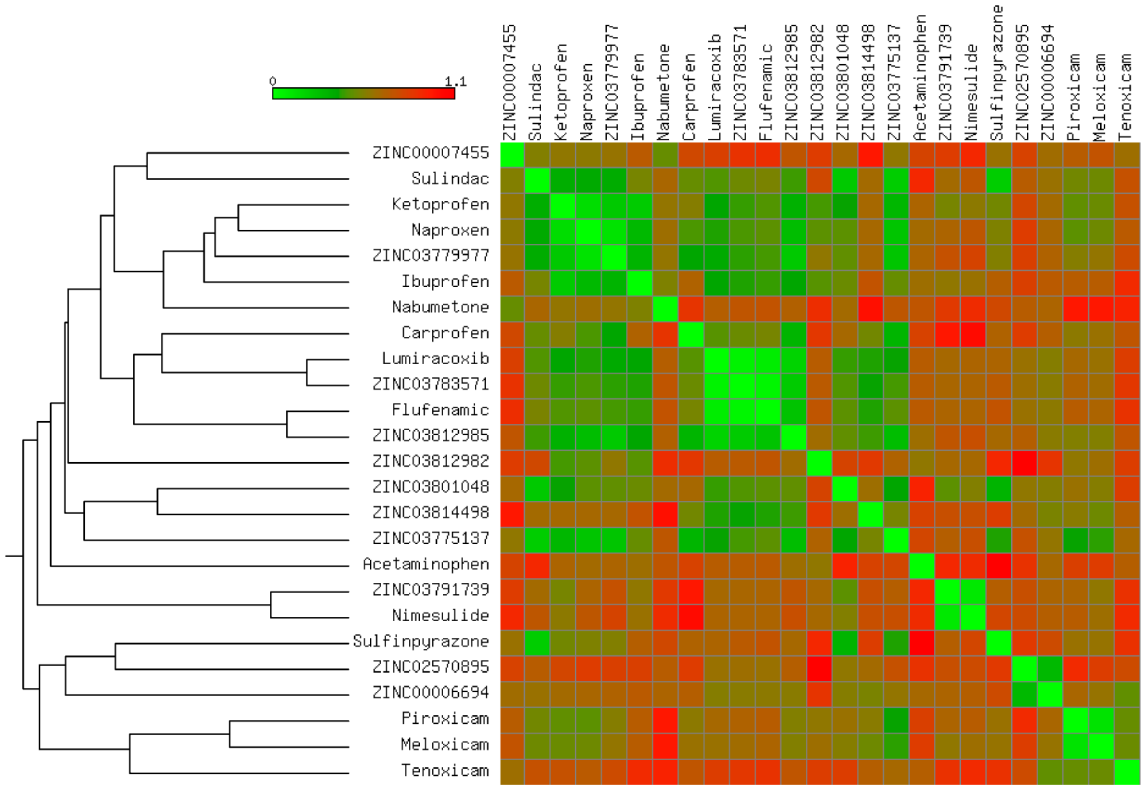
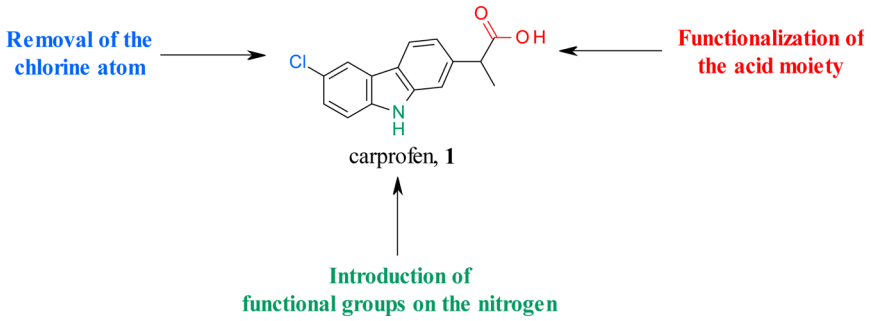


Figure 2. Tree based on the pairwise Tanimoto fingerprint distances between the 25 COX inhibitors tested in the present study. The heat map highlights the distances calculated in the first 5 principal components space (variance explained >90%) originating from 10 physicochemical descriptors (i.e., net charge, MW, log *P*, log *S*, number of hydrogen bond donors, number of hydrogen bond acceptors, polar surface area, number of atoms, number of rings, and number of rotatable bonds).

Scheme 1. Planned Chemical Variations of Carprofen, 1



Chemistry. Several possible chemical variations of **1** were considered (Scheme 1). The preparation of the corresponding deschlorinated derivative (**2**) was performed by hydrogenation of **1**, using a flow hydrogenator apparatus (Scheme 2).

Syntheses of the ester and amide derivatives of **1** were achieved using standard reaction conditions (Scheme 2, for representative examples). The esterification of **1** in acidic methanol gave the corresponding methyl ester **3** in quantitative yield. The amides **4a** and **4b** were efficiently prepared by the reaction of **1** in pyridine in the presence of CDI with ethanolamine and aniline, respectively.

The functionalization of the nitrogen atom of **1** was achieved using ester **3** as a common intermediate (Scheme 3). The preparation of different *N*-alkyl derivatives was performed using the appropriate alkyl halides in the presence of Cs_2CO_3 in MeCN to provide compounds **5** in moderate to good yields. Saponification of the ester with LiOH, followed by acidic treatment, gave the corresponding carboxylic acids **6** in good yields.

Preparation of the sulfonamide derivatives **7** was achieved by reaction of **3** with the appropriate sulfonyl chlorides in THF, in the presence of DMAP and Et_3N, under thermal heating or microwave irradiation. In a similar manner, as described before, saponification of the ester group produced the corresponding acid **8** in good yields.

Reaction of compound **3** with the appropriate isocyanates in THF, in the presence of DMAP and Et_3N, under microwave conditions, afforded the urea derivatives **9**. Compounds **9** were then hydrolyzed under basic conditions to give the corresponding acid derivatives **10** in moderate to good yields. In the case of **9d**, bearing a *p*-chlorophenyl moiety, the methyl ester hydrolysis was performed in acidic medium (due to the observed cleavage of the urea bond of **9d** under basic conditions) to provide **10d** in a good 80% yield.

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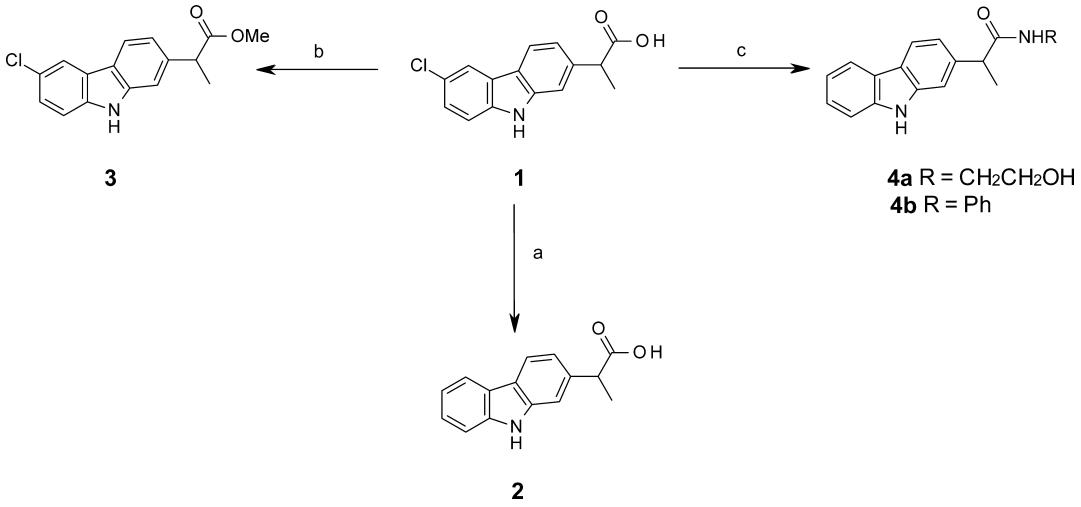
Scheme 2. Synthesis of Deschlorinated Compound 2, Ester 3, and Amides 4[a]



[a]Reagents and conditions: (a) H-Cube, H_2, 10% Pd/C, EtOH, EtOAc, 60 °C, 40%; (b) MeOH, H_2SO_4, rt, 12 h, quantitative; (c) CDI, pyridine, 60 °C, 12 h, 85−86%.

The carbamate derivative **12** was prepared under standard conditions (Scheme 3). Compound **3** was reacted in THF with hexyl chloroformate, in the presence of Et_3N and DMAP, to give intermediate **11**, which was further hydrolyzed under acidic conditions to provide **12**.

The preparation of the acyl derivatives was performed using a different protecting group on the carboxylic functionality (Scheme 4). In our initial experiments, it was indeed observed that *N*-acetylated derivatives were not stable under basic and acidic conditions, typically used for the hydrolysis of the methyl ester. The use of *tert*-butyl ester was also exploited without any synthetic success.[42] We therefore decided to protect **1** as an *O*-benzyl ester, using standard reaction conditions (BnBr, K_2CO_3, DMF).[43] The acylation reactions of the benzyl ester **13** proceeded well in MeCN in the presence of DMAP and Et_3N, with various acyl chlorides, to afford esters **14**.

The last debenzylation step to obtain the corresponding acids **15** was performed using the H-Cube apparatus, under optimized conditions to reduce the formation of the undesired deschloro derivatives **16**. In this context, the influence of the solvent (EtOH/EtOAc (1:1) and THF), the temperature of the reaction (from room temperature to 60 °C), the catalyst loading (1%, 5%, and 10% Pd/C cartridge), and the hydrogenation conditions were evaluated. After several trials, two different conditions appeared to be satisfactory and were applied for the preparation of our targeted compounds. The first method relied on passing a solution of **14** in THF through a 5% Pd/C cartridge, at room temperature and 1 bar of H_2 pressure (H-Cube "full H_2 mode"). In this case, complete conversion of the benzyl ester **14** was observed, and the expected **15** was formed in an approximately 3:1 ratio with respect to **16**. The second method was performed using a 1% Pd/C cartridge at room temperature and 1 bar of H_2 pressure, in a closed-loop system with the H-Cube (where the outcoming solution was directly reinjected into the incoming solution). By carefully monitoring the advancement of the reaction by UPLC/MS analysis, it was possible, after 4−5 h of hydrogenation, to reach a stage where the conversion of **14** was satisfactory (generally around 70%) and where the formation of **16** was maintained low compared to that of **15**; the reaction was stopped when the **15**:**16** ratio reached approximately 9:1. **15a−j** were prepared using this procedure with the exception of **15k**. In this case, we observed that the final hydrogenation step did not proceed, probably because of the presence of the thiazole ring, which poisoned the catalyst. We therefore decided to use the methyl ester **3** as the starting material and to carry the hydrolysis in acidic medium, which led to the formation of **15k** in a low yield.

Structure−Activity Relationship (SAR) Exploration. The inhibitory activities against FAAH, COX-1, and COX-2 of the first set of derivatives of **1** are summarized in Table 1. The IC_{50} values were not determined for compounds showing less than 50% inhibition at concentrations of 100 μM for FAAH and COXs.

Compound **1** inhibited FAAH, COX-1, and COX-2 with a rather balanced profile (Table 1). Although an improved potency against FAAH was observed with **2** (IC_{50} = 5 μM), the removal of the chlorine atom was detrimental for the inhibition of both COX-1 and COX-2. Also, the pivotal role of the carboxylic function for COX inhibition was confirmed by the lack of activity of the ester derivative **3** and the amides **4a** and **4b**. On the other hand, we observed a 26-fold increase in FAAH potency for **4b** (IC_{50} = 3 μM) compared to **1**. On the basis of these results, we decided to continue the exploration exclusively on derivatives bearing both the chlorine atom and the carboxylic function.

We investigated the functionalization of the nitrogen atom of the carbazole core. The *N*-alkylated compounds **6a−g** and the *N*-arylsulfonylated derivatives **8a**,**b** were devoid of COX inhibitory activity. On the other hand, the compounds showed a slightly better FAAH inhibitory potency compared to **1**. The introduction of a methyl group (**6a**) led to an IC_{50} = 15 μM against FAAH, while a benzyl group on the carbazole nitrogen gave a 3-fold improvement in FAAH inhibition compared to that of **1** (**6b**, IC_{50} = 23 μM). We also evaluated the influence of the substitution on the phenyl ring. Also compound **6c**, which bears a CN group in the *para* position, showed a slight decrease in FAAH potency (IC_{50} = 33 μM). Conversely, the *p*-Cl derivative **6d** and the *p*-OCH_3 derivative **6e** showed greater potency against FAAH, with IC_{50} values of 10 and 8 μM, respectively. Moreover, we observed that the *para* substitution was preferential: the *p*-OCH_3 derivative **6e** was more potent

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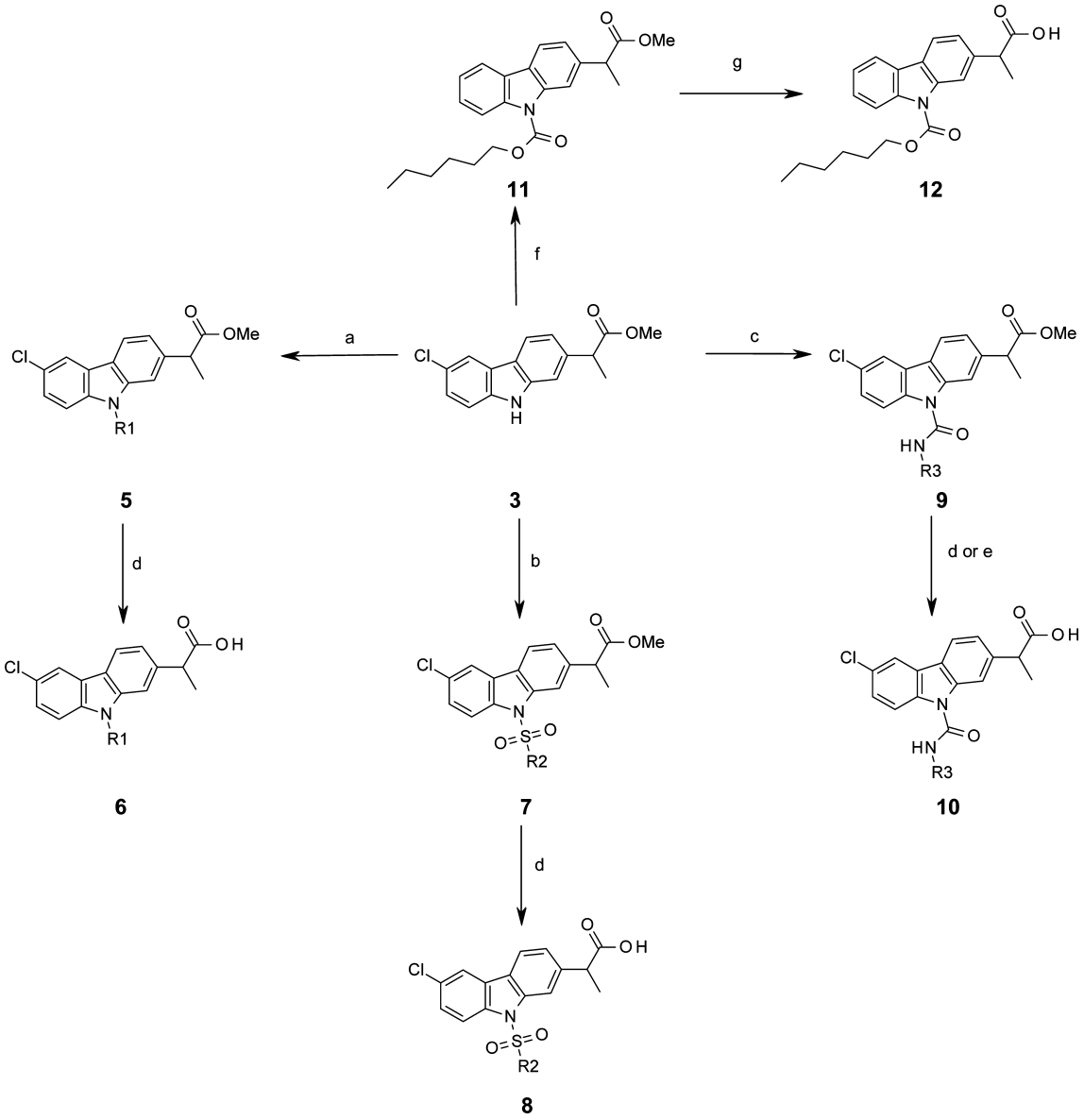
Scheme 3. Synthesis of Compounds 6, 8, 10, and 12[a]



[a]Reagents and conditions: (a) R_1X, Cs_2CO_3, MeCN, reflux, 12 h, 32−99%; (b) R_2SO_2Cl, Et_3N, DMAP, THF, reflux, 5 h, or 100 °C, 3 h, microwave, 35−78%; (c) R_3NCO, Et_3N, DMAP, THF, 100 °C, microwave, 10 h, 51−81%; (d) LiOH, MeOH, THF, H_2O, 12 h, 21−85%; (e) 6 M HCl, THF, rt, 5 days, 80%; (f) hexyl chloroformate, Et_3N, DMAP, THF, 100 °C, 3 h, microwave, 85%; (g) 6 M HCl, THF, rt, 3 days, 55%.

against FAAH (IC_{50} = 8 μM) than the *m*-OCH_3 derivative **6f** (IC_{50} = 32 μM) and the *o*-OCH_3 derivative **6g** (which showed no activity at 30 μM). Within the series of sulfonamides, **8a** and **8b** were active against FAAH, displaying IC_{50} values of 50 and 5 μM, respectively. It is worth mentioning that *N*-alkylated and *N*-arylsulfonylated carprofen derivatives were previously investigated for their ability to inhibit γ-secretase activity.[43]

A number of urea derivatives were also investigated. Here, too, it was observed that inhibitory activity was generally lost on both COX isoforms, while FAAH inhibitory activity was highly dependent on the type of substituent on the urea moiety. Indeed, the best FAAH inhibitor of this series was **10a** (IC_{50} = 3 μM), bearing a linear C_6 chain. Shorter linear chains, from C_1 to C_4, showed no inhibition on FAAH, as exemplified by **10b** and **10c**. Longer chains brought less potent FAAH inhibitors, but interestingly led to an increase in COX-1 inhibition. For example, **10d** showed an IC_{50} = 27 μM against FAAH and an IC_{50} = 15 μM against COX-1. Compound **10e** (bearing a 4-chlorobenzyl moiety) showed weak potency against FAAH (IC_{50} = 102 μM) and an IC_{50} value of 41 μM against COX-1. *N*,*N*-Disubstituted derivatives or ureas bearing a cyclic moiety, such as **10f** and **10g**, were also prepared, but they all did not show any improved activities against the targets. Compound **12** showed a 10-fold weaker potency against FAAH (IC_{50} = 32 μM) compared to its carbamate analogue **10a** (IC_{50} = 3 μM), while it showed an IC_{50} = 17 μM on COX-1.

We then analyzed a set of acyl derivatives. The acetyl derivative **15a** did not show any inhibitory potency. Compound **15b** was active only against FAAH (IC_{50} = 25 μM). Compound **15c**, which bears a *p*-chlorobenzoyl group, showed promising inhibitory potency on FAAH (IC_{50} = 22 μM) and COX (COX-1 IC_{50} = 74 μM and COX-2 IC_{50} = 72 μM).

In light of these promising results, we came to the conclusion that a carbonyl function linked to the nitrogen atom of the

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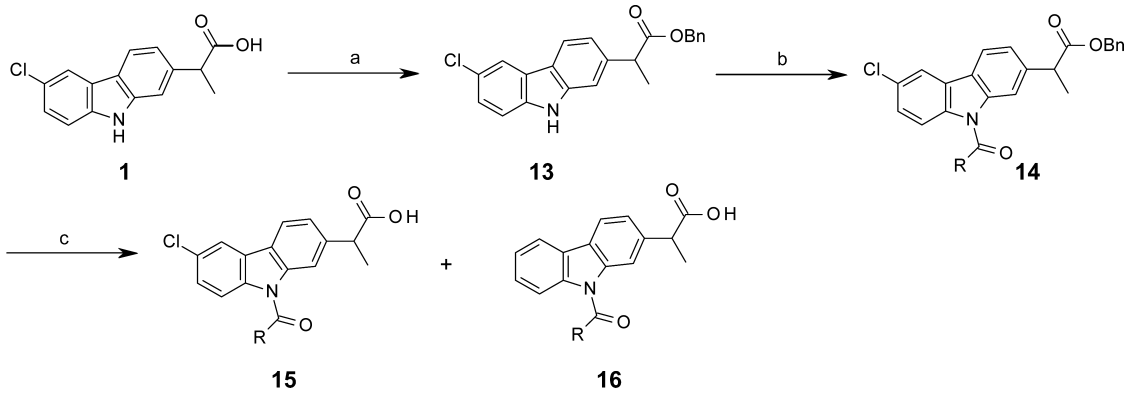
Scheme 4. Synthesis of Compounds 15[a]



[a]Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 3 h, 84%; (b) acyl chloride, DMAP, Et_3N, MeCN, rt, 2 h, 76–97% ; (c) H-Cube, H_2, 1% or 5% Pd/C, rt, THF, 15–42%.

carbazole ring of **1** could be important to obtain a multitarget inhibition. Therefore, we decided to further expand the SAR around **15c**, obtaining a second series of molecules, whose activities are summarized in Table 2.

Focusing on the benzoyl core of **15c**, we prepared the *p*-fluorobenzoyl and *p*-methoxybenzoyl analogues **15d** and **15e**, which showed IC_{50} values on FAAH of 31 and 11 μM, respectively. However, the compounds did not inhibit COX-1 or COX-2 activity. We then analyzed the influence of the position of the chlorine atom on the phenyl ring. Interestingly, as we reported before in the *N*-alkyl series, the *para* substitution was the most tolerated. The *m*-Cl derivative **15f** (FAAH IC_{50} = 20 μM) and the *o*-Cl derivative **15g** (FAAH IC_{50} = 60 μM) retained a similar inhibitory potency for FAAH compared to **15c**, while inhibitory activity against COX was lost. A total loss of activity for the three enzymes was observed when we tried to combine the most tolerated *p*- and *m*-Cl substitutions, as shown with **15h**.

The inhibitory activity on the three enzymes was also highly dependent on the nature of the heteroaromatic ring. The 4-oxazole substitution of **15i** proved to be well tolerated, with FAAH IC_{50} = 85 μM, COX-1 IC_{50} = 30 μM, and COX-2 IC_{50} = 28 μM. Compound **15j**, bearing a 4-imidazole ring, showed FAAH IC_{50} = 6 μM and COX-1 IC_{50} = 13 μM, but no relevant inhibition of COX-2. The introduction of a 4-thiazole ring in **15k** was detrimental for the inhibition of FAAH and COX enzymes.

We finally analyzed the potency of the single enantiomers of the most active compounds of the present series, namely, **1**, **15c**, and **15i**. The racemic mixtures were submitted to enantiomeric separation by chiral HPLC. The results obtained are summarized in Table 3. For **1**, the (*S*)-(+) enantiomer was the only one active against the three targets. For **15c** and **15i**, the (*S*)-(+) enantiomer was the only one active against COXs while the inhibitory activity against FAAH was obtained with the (*R*)-(−) enantiomer for both compounds. These findings are consistent with the known ability of the (*R*) enantiomers of flurbiprofen, indomethacin, and celecoxib to inhibit FAAH.[44,45] In addition, the inactivity of (*R*)-(−) enantiomers of **1**, **15c**, and **15i** against COXs was expected, in agreement with previous results showing a preferential COX inhibition by (*S*)-enantiomers.[46]

DISCUSSION

The present results identify carprofen (compound **1**) as a multitarget FAAH/COX inhibitor. SAR studies on the scaffold of the molecule show that different functionalizations at the nitrogen atom of the carbazole ring of this molecule yield additional active compounds, such as **15c** and **15i**, which inhibit FAAH/COX activities in the low micromolar range (see Tables 1 and 2). The ability of NSAIDs to inhibit anandamide hydrolysis has been previously described by Fowler and co-workers in several informative studies.[39,47,48] These investigators described new molecules that simultaneously target FAAH and COX activities. In particular, the compound ibu-am5 (*N*-(3-methylpyridin-2-yl)-2-(4-isobutylphenyl)-propionamide) was shown to be a promising multitarget FAAH/COX inhibitor.[23,24,39,49] This compound displayed an IC_{50} value for FAAH of ∼0.5 μM (in EtOH) and IC_{50} values for COX-1 of ∼60 μM and ∼240 μM in DMSO and ethanol, respectively. These data for COX inhibition were generated using an oxygen electrode assay for peroxidase (POX) activity.[24] Under these experimental conditions, ibu-am5 was inactive against COX-2. However, if anandamide was used as a COX-2 substrate in the assay, instead of arachidonic acid, ibu-am5 showed a COX-2 $IC_{50} \approx 20$ μM, suggesting a substrate dependency in COX-2 inhibition.[24] Under our present experimental conditions, ibu-am5 inhibited FAAH with an IC_{50} of 66 μM. COX inhibition was measured with an enzyme immunoassay using a prostaglandin-specific antibody (see the Methods), which yielded an IC_{50} for COX-1 of 170 μM. The potency toward COX-2 was very low, as ibu-am5 produced only 30% inhibition at 30 μM. We also tested ibuprofen as an additional reference compound, which showed IC_{50} values of 5 and 36 μM against COX-1 and COX-2, respectively, while it was very weak against FAAH, in reasonably good agreement with previous findings.[47,50]

Therefore, carprofen and its racemic derivatives described in the present study are among the most potent multitarget FAAH/COX inhibitors reported so far. These molecules also show a well-balanced multitarget profile, implying that they may provide a starting point for the discovery of new FAAH/COX inhibitors to treat pain, inflammation, and potentially, as recently discussed, cancer.[51,52] In addition, these compounds may provide a pharmacological tool to characterize the synergistic effect produced by the simultaneous inhibition of FAAH and COXs in different pathological conditions.[34]

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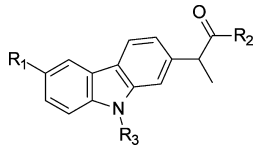
Table 1. FAAH-1, COX-1, and COX-2 Activities of Carprofen Derivatives[a]



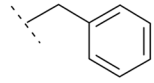
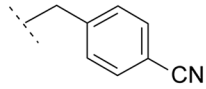
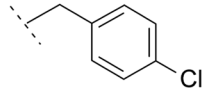
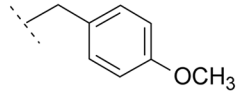
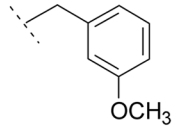
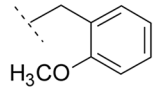
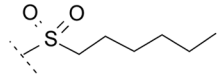
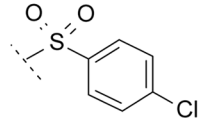
Compd	R_1	R_2	R_3	IC_{50} (μM) ± SD		
				FAAH	COX-1	COX-2
1	Cl	OH	H	78.6±19.7	22.3±6.6	3.9±1.0
2	H	OH	H	4.6±0.3	>100	>100
3	Cl	OCH_3	H	22.1±2.4	>100	>100
4a	Cl	$HOCH_2CH_2NH$	H	>100μM	>100	>100
4b	Cl	C_6H_5NH	H	2.9±0.2	>100	>100
6a	Cl	OH	CH_3	15.5±2.5	>100	>100
6b	Cl	OH		23.3±2.5	>100	>100
6c	Cl	OH		33.4±15.7	>100	>100
6d	Cl	OH		10.2±0.9	>100	>100
6e	Cl	OH		8.4±2.4	>100	>100
6f	Cl	OH		32.5±7.1	>100	>100
6g	Cl	OH		>100	>100	>100
8a	Cl	OH		50.3±2.3	>100	>100
8b	Cl	OH		4.8±4.5	>100	>100

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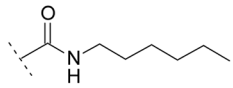
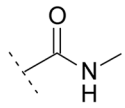
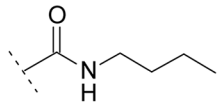
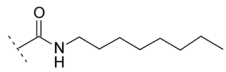
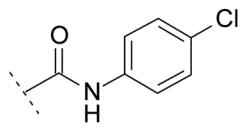
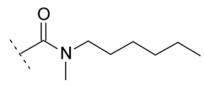
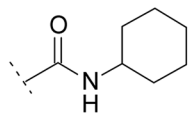
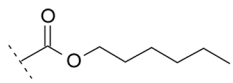
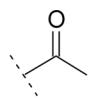
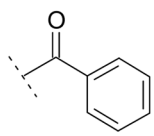
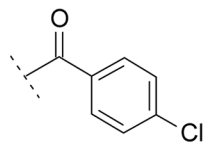
Table 1. continued

Compd	R_1	R_2	R_3	IC_{50} (μM) ± SD		
				FAAH	COX-1	COX-2
10a	Cl	OH		3.2±1.7	>100	>100
10b	Cl	OH		>100	>100	>100
10c	Cl	OH		>100	>100	>100
10d	Cl	OH		26.8±8.1	15.4±8.8	>100
10e	Cl	OH		102.0±6.1	41.3±6.9	>100
10f	Cl	OH		>100	>100	>100
10g	Cl	OH		>100	>100	>100
12	Cl	OH		31.6±0.8	17.2±1.9	>100
15a	Cl	OH		>100	>100	>100
15b	Cl	OH		25.4±1.9	>100	>100
15c	Cl	OH		22.0±4.2	74.3±28.0	72.3±28.0

[a]Values are means of ≥3 experiments performed in duplicate.

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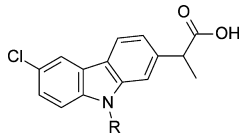
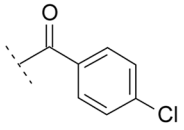
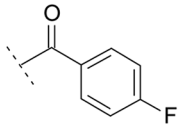
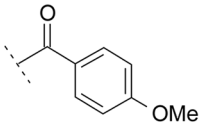
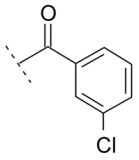
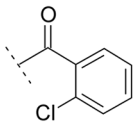
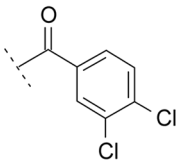
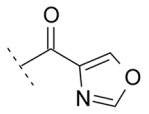
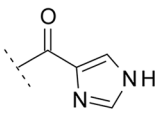
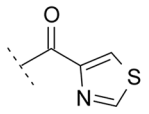
Table 2. SAR Exploration around 15c[a]



Compd	R	IC_{50} (μM) ± SD[b]		
		FAAH	COX-1	COX-2
15c		22.0±4.2	74.3±28.0	72.3±28.0
15d		30.9±10.6	>100	>100
15e		10.6±2.6	>100	>100
15f		20.1±2.6	>100	>100
15g		59.6±15.5	>100	>100
15h		>100	>100	>100
15i		84.8±10.6	30.0±12.1	27.8±9.7
15j		5.6±2.9	12.8±8.9	>100
15k		>100	>100	>100

[a]Values are means of ≥3 experiments performed in duplicate.

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Table 3. FAAH, COX-1, and COX-2 Activities of Single Enantiomers of Compounds 1, 15c, and 15i[a]

	IC_{50} (μM) ± SD		
compd	FAAH	COX-1	COX-2
(±)-**1**	78.6 ± 19.7	22.3 ± 6.6	3.9 ± 1.0
(*S*)-(+)-**1**	64.2 ± 3.6	5.6 ± 0.1	5.3 ± 3.0
(*R*)-(−)-**1**	>100	>100	>100
(±)-**15c**	22.0 ± 4.2	74.3 ± 28.0	72.3 ± 28.0
(*S*)-(+)-**15c**	>100	45.0 ± 0.3	46.5 ± 4.3
(*R*)-(−)-**15c**	14.9 ± 1.6	>100	>100
(±)-**15i**	84.8 ± 10.6	30.0 ± 12.1	27.8 ± 9.7
(*S*)-(+)-**15i**	>100	4.1 ± 2.8	2.5 ± 1.4
(*R*)-(−)-**15i**	53.2 ± 22.6	>100	>100

[a]Values are means of ≥3 experiments performed in duplicate.

Despite being evolutionary unrelated, FAAH and COXs share several structural similarities, which might help in the rationalization of the multitarget activity of carprofen and its derivatives. Such common features of the catalytic pocket of FAAH and COXs could also be anticipated in light of the similarity of their endogenous substrates. The main product of FAAH activity on anandamide is arachidonic acid, which is a substrate for COXs. In addition, it has been shown that anandamide can be metabolized by COX-2.[49] Because of the presence of such common structural features, useful also to discuss the basis of biological promiscuity,[53] we mapped FAAH and COX-2 with two probes able to pinpoint hydrophilic and hydrophobic spots of the proteins (Figure 3). The acyl chain binding channel is the most hydrophobic portion of FAAH, while, in proximity of the oxyanion hole, the hydrophilic features become preponderant due to both the presence of hydroxyl groups and the proximity of the cytosolic channel. We found a similar pattern in COX-2. In the case of this enzyme, the core of the binding site is mainly hydrophobic. In fact, this is the volume that hosts the lipophilic core of virtually all known COX inhibitors. Approaching the exit of the site, the hydrophilic character increases. The polar tails of several well-known COX inhibitors lie in this area, establishing H-bond interactions with Arg120 and Tyr355. The most important difference can be observed for the hydrophobic volume, which is greater in the case of FAAH (Figure 3).

On the basis of our docking results, we are quite confident in the binding mode of **1** in COXs, since it is consistent with the experimentally observed binding mode of several arylpropionic acid derivatives in complex with either of the two COX isoforms.[9] On the other hand, in FAAH, **1** could be accommodated into the acyl chain binding channel and establish H-bond interactions with the oxyanion hole through its carboxylic group. However, we cannot rule out alternative binding modes, in different regions of the large binding cavities of FAAH's catalytic site (see Figure S3, Supporting Information). Notably, **1** is a small molecule bearing fragment-like physicochemical features.[54]

Finally, it is worth mentioning that the multitarget FAAH/COX activity described in this study could in principle explain why **1** is reported to have reduced ulcerogenic side effects in humans compared to other NSAIDs.[55] The ability of this compound to block FAAH activity might attenuate the ulcerogenic effects of COX inhibition, as shown in mice for the combination URB937 plus indomethacin.[34]

CONCLUSIONS

We reported on the identification of multitarget inhibitors that simultaneously block FAAH, COX-1, and COX-2 activities. The concomitant inhibition of these enzymes has recently been shown to produce, in vivo, improved analgesic response and diminished side effects in animal models of pain. Despite their still moderate activity, the present series of compounds comprise the most active multitarget FAAH/COX inhibitors reported in the literature so far. They could serve, therefore, both as starting points for future drug discovery efforts and as tools to further characterize the synergistic effects obtained by the simultaneous inhibition of FAAH and COX activities.

EXPERIMENTAL SECTION

COX Inhibitor Data Set. A large collection of known COX inhibitors was retrieved, in a ready-to-dock format, from DUD[41] and from DrugBank.[40] The grand total of unique molecules collected, after elimination of double occurrences, was 382. Some of these are currently marketed drugs mostly in use as anti-inflammatory agents,

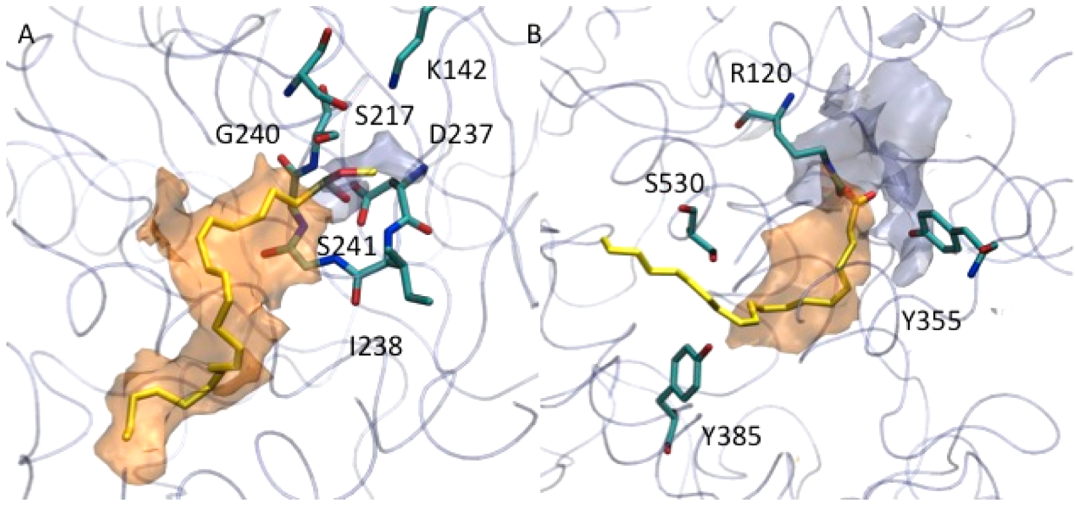

Figure 3. Hydrophilic (light blue) and hydrophobic (orange) isocontour surfaces of FAAH-1 (A) and COX-2 (B). For the sake of clarity, relevant residues are highlighted as stick models with C atoms colored in cyan. The protein is shown as a transparent cyan tube. Substrates are methyl arachidonyl fluorophosphonate in FAAH and arachidonic acid in COX-2.

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while others have been reported to inhibit at least one of the two COX isoforms, with diverse potencies. For years, COX inhibitors have been classified according to their binding preferences and mechanism of action. A clear-cut distinction between classes can be sometimes misleading and formally incorrect. In general, there can be selective, nonselective, and isoenzyme-preferring binders that can act through (i) an irreversible mechanism (e.g., aspirin), (ii) a reversible substrate competitive binding (e.g., piroxicam), (iii) a slow, time-dependent noncovalent action (e.g., indomethacin), and (iv) a slow, time-dependent COX-2 irreversible type of inhibition (e.g., rofecoxib/Vioxx, celecoxib/Celebrex). Very recently, Marnett and colleagues showed that the (*R*) enantiomers of several well-known COX inhibitors such as naproxen, flurbiprofen, and ibuprofen, considered to be COX-2 inactive, are, in fact, "substrate-selective inhibitors".[56]

The data set reported here comprises all the classes of inhibitors mentioned above, among which we can find classic NSAIDs, such as Fenac (e.g., sulindac) and Profen derivatives (e.g., ketoprofen), oxicams (e.g., tenoxicam), and some classes of COX-2-selective binders, such as di(hetero)aryl (thio)ethers (e.g., NS-398, nimesulide), carbocycles and heterocycles with vicinal aryl substitutions (e.g., valdecoxib, celecoxib, and the recently retired from the market xib), just to mention a few. However, the collection presented here is meant to be a set of readily accessible molecules known to inhibit at least one of the two COX isoforms and to be used as starting points toward the search for dual FAAH/COX inhibitors, rather than being an exhaustive collection of COX inhibitors.

The entire assembled set was clustered according to pairwise Tanimoto distances, using a description based on the Daylight fingerprints (Figure 1). A clustering threshold of 0.4 resulted in 84 clusters, which highlighted the structural diversity within the set. The chemotype richness found among the selected COX inhibitors is convenient when looking for binders of an evolutionary unrelated target (i.e., FAAH), as in this case. To better characterize the molecular diversity in the data set, we looked at specific physicochemical features of each compound. The distribution observed for nine common physicochemical descriptors further describes the diversity found over the entire data set. Normal distributions can be observed for most of the selected descriptors. An exception was the total charge descriptor. In fact, apart from a few negatively charged molecules, the vast majority of entries were neutral. Also, most compounds showed a number of hydrogen bond donors of either 0 or 2 (see Figure S1, Supporting Information).

Molecular Descriptors and Fingerprint Similarity. Molecular properties for each fragment were calculated by means of ICM3.7 (Molsoft LLC, San Diego, CA). Molecular patterns were calculated and hashed into bitmaps according to the Daylight algorithm for fingerprint generation (Daylight Chemical Information Systems Inc., Laguna Niguel, CA) as available in ICM3.7 (Molsoft LLC, San Diego, CA). Similarity between molecules was calculated as the difference between 1 and the Tanimoto coefficient, $T_c = c/(a + b - c)$, where c counts the common bits *on* molecule 1 and molecule 2, a counts the bits *on* molecule 1, and b counts the bits *on* molecule 2. T_c spans from 0 to 1, with 1 indicating that two molecules share the same fingerprint.

Docking and Binding Site Mapping. Chain A of the X-ray structure of rFAAH, covalently bound to a molecule of methyl arachidonyl fluorophosphonate, as available at the Protein Data Bank (PDB ID 1mt5),[57] and chain A of the X-ray structure of COX-2, in complex with flurbiprofen (PDB ID 3pgh),[58] were used for the docking study. Missing side chains were added through SCWRL 4.0.[59] Then water and cocrystallized ligands were removed, and the catalytic serine (S241) was treated as negatively charged. The so-obtained structures were energy minimized with the NAMD 2.6[60] program until a 0.3 Å rmsd convergence criterion on heavy atoms was reached. The Amber force field parm99SB[61] was employed throughout. The final protein models and the selected known COX inhibitors were then converted through the Python scripts available in the ADT suite of programs[62] into a suitable format for AutoDock Vina v.1.0.[63]

AutoDock Vina v.1.0[63] was used to dock 382 known COX inhibitors at the FAAH binding site. The search volume, centered on the phosphate atom of the cocrystallized inhibitor, spanned 24 Å in the three directions. The algorithm search exhaustiveness was set to 4, and the maximum number of output poses was set to 10. Finally, the top 100 ranked molecules (approximately 25% of the distribution) were visually inspected to eliminate hits that did not directly bind in proximity of the oxyanion hole. We checked the commercial availability of each of the top 100 scored molecules and eventually purchased 25 compounds for experimental testing (see Figure 2). Some molecules such as acetaminophen and aspirin that exceeded the rank threshold (rank nos. 350 and 326, respectively) were considered because they were readily available. Despite their low scores, we also included molecules belonging to an under-represented chemical class, the oxicams (i.e., piroxicam, meloxicam, and tenoxicam). When a top-ranked molecule was not available, we purchased alternative molecules that were close in the Tanimoto fingerprint space, regardless of their estimated binding energies. For example, diclofenac and etodolac were purchased in place of ZINC03814788 (rank no. 61) and ketorolac (rank no. 53), respectively.

General Methods for Synthesis. Solvents and reagents were obtained from commercial suppliers and were used without further purification. For simplicity, solvents and reagents are indicated as follows: acetyl chloride (AcCl), acetonitrile (MeCN), ammonium chloride (NH_4Cl), benzyl bromide (BnBr), carbonyldiimidazole (CDI), cesium carbonate (Cs_2CO_3), cyclohexane (Cy), chloroform ($CHCl_3$), dichloromethane (DCM), dimethyl sulfoxide (DMSO), diethyl ether (Et_2O), *N*,*N*-diisopropylethylamine (DIPEA), 4-(dimethylamino)pyridine (DMAP), di-*tert*-butyl dicarbonate (Boc_2O), ethanol (EtOH), ethyl acetate (EtOAc), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), iodomethane (MeI), *N*,*N*-dimethylformamide (DMF), lithium hydroxide (LiOH), magnesium sulfate ($MgSO_4$), methanol (MeOH), sodium bicarbonate ($NaHCO_3$), tetrabutylammonium iodide (TBAI), tetrahydrofuran (THF), triethylamine (Et_3N), trifluoroacetic acid (TFA).

Automated column chromatography purifications were done using a Teledyne ISCO apparatus (CombiFlash Rf) with prepacked silica gel columns of different sizes (from 4 to 120 g). Mixtures of increasing polarity of cyclohexane and ethyl acetate or dichloromethane and methanol were used as eluents. Preparative TLC were performed using Macherey-Nagel precoated 0.05 mm TLC plates (SIL G-50 UV_{254}). Hydrogenation reactions were performed using H-Cube continuous hydrogenation equipment (SS-reaction line version), employing disposable catalyst cartridges (CatCart) preloaded with the required heterogeneous catalyst. Microwave heating was performed using the Explorer-48 position instrument (CEM). NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for ^1H and 100.62 MHz for ^{13}C), equipped with a BBI probe and *Z*-gradients. Spectra were acquired at 300 K, using deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated chloroform ($CDCl_3$) as the solvent. Chemical shifts for ^1H and ^{13}C spectra were recorded in parts per million using the residual nondeuterated solvent as the internal standard (for DMSO-d_6, 2.50 ppm, ^1H, and 39.52 ppm, ^{13}C; for $CDCl_3$, 7.26 ppm, ^1H, and 77.16 ppm, ^{13}C). Data are reported as follows: chemical shift (ppm), multiplicity (indicated as br, broad signal, s, singlet, d, doublet, t, triplet, q, quartet, p, quintet, sx, sextet, and m, multiplet, and combinations thereof), coupling constant (*J*, Hz), and integrated intensity. UPLC/MS analyses were run on a Waters ACQUITY UPLC/MS system consisting of an SQD (single-quadropole detector) mass spectrometer equipped with an electrospray ionization interface and a photodiode array detector (PDA). The PDA range was 210−400 nm. Analyses were performed on an ACQUITY UPLC BEH C18 column (50 × 2.1 mm i.d., particle size 1.7 μm) with a VanGuard BEH C18 precolumn (5 × 2.1 mm i.d., particle size 1.7 μm). The mobile phases were either 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in MeCN−H_2O (95:5) at pH 5 (B) or 5 mM NH_4OAc + 0.25% AcOH in H_2O (A) and 5 mM NH_4OAc + 0.25% AcOH in MeOH (B) for compounds **5**. Electrospray ionization in positive and negative modes was applied. Purifications by preparative HPLC/MS were run on a Waters Autopurification system consisting of a 3100 single-quadropole mass spectrometer equipped with an electrospray ionization interface and a 2998 photodiode array detector. The HPLC system included a 2747 sample manager, 2545

binary gradient module, system fluidic organizer, and 515 HPLC pump. The PDA range was 210−400 nm. Purifications were performed on a XBridge Prep C18 OBD column (100 × 19 mm i.d., particle size 5 μm) with an XBridge Prep C18 (10 × 19 mm i.d., particle size 5 μm) guard cartridge. The mobile phases were 10 mM NH_4OAc in H_2O at pH 5 adjusted with AcOH (A) and 10 mM NH_4OAc in MeCN-H_2O (95:5) at pH 5 (B). Electrospray ionization in positive and negative modes was used. Analyses by chiral HPLC were run on a Waters Alliance HPLC instrument consisting of an e2695 separation module and a 2998 photodiode array detector. The PDA range was 210−400 nm. Analyses were performed isocratic on a Daicel ChiralPak AD column (250 × 4.6 mm i.d., particle size 10 μm). The mobile phase was 0.1% TFA heptane/2-propanol (75:25). Separations by preparative chiral HPLC were run on a Waters Alliance HPLC instrument consisting of a 1525 binary HPLC pump, a Waters Fraction Collector III, and a 2998 photodiode array detector. UV detection was at 240 nm. Purifications were performed isocratic on a Daicel ChiralPak AD column (250 × 10 mm i.d., particle size 10 μm). The mobile phase was 0.1% TFA heptane/2-propanol (75:25). Optical rotations were measured on a Rudolf Research Analytical Autopol II automatic polarimeter using a sodium lamp (589 nm) as the light source (concentrations expressed in grams per 100 mL using EtOAc or MeOH as the solvent and a 1 dm cell. All final compounds displayed ≥95% purity as determined by NMR and UPLC/MS analysis.

2-(9*H*-Carbazol-2-yl)propanoic Acid (2). A solution of carprofen (**1**, 106 mg, 0.39 mmol) in a 1:1 mixture of EtOH/EtOAc (20 mL) was hydrogenated with the ThalesNano H-Cube using a 10% Pd/C catalyst, at a 1 mL/min flow rate and room temperature and in a H_2 atmosphere (1 atm) (full H_2 mode). After three consecutive runs, the solvent was removed in vacuo, and the crude oil was purified by preparative HPLC to yield the corresponding deschlorinated compound **2** (37 mg, 40%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 7.09 (dd, J = 8.1, 1.3 Hz, 1H), 7.14 (m, 1H), 7.38 (m, 2H), 7.47 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 11.18 (s, 1H), 12.25 (s, 1H); MS (ES, m/z) $C_{15}H_{13}NO_2$ requires 239, found 240 $[M + H]^+$.

Methyl 2-(6-Chloro-9*H*-carbazol-2-yl)propanoate (3). To a suspension of **1** (1.055 g, 3.85 mmol) in MeOH (40 mL) was added H_2SO_4 (0.1 mL). The mixture became homogeneous and was stirred at room temperature overnight. After evaporation of MeOH, the residue was taken up in EtOAc and washed with a saturated aqueous $NaHCO_3$ solution (3 × 20 mL) and brine (10 mL). After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo to give **3** (1.15 g, quantitative) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59 (d, J = 7.2 Hz, 3H), 3.68 (s, 3H), 3.88 (q, J = 7.2 Hz, 1H), 7.18 (dd, J = 8.1, 1.4 Hz, 1H), 7.37 (m, 3H), 7.95 (d, J = 8.1 Hz, 1H), 7.99 (m, 1H), 8.02 (s, 1H); MS (ES, m/z) $C_{16}H_{14}ClNO_2$ requires 287, found 286 $[M - H]^-$.

2-(6-Chloro-9*H*-carbazol-2-yl)-*N*-(2-hydroxyethyl)-propanamide (4a). To a solution of **1** (112 mg, 0.41 mmol, 1 equiv) in pyridine (2 mL) was added CDI (133 mg, 0.82 mmol, 2 equiv) portionwise. The mixture was stirred for 2 h at room temperature, before addition of ethanolamine (50 μL, 0.82 mmol, 2 equiv), and then the reaction was heated at 55 °C overnight. Pyridine was then evaporated and the residue taken up in EtOAc and washed with a saturated aqueous NH_4Cl solution (5 mL). After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (DCM/MeOH) to give amide **4a** (110 mg, 85%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.41 (d, J = 7.0 Hz, 3H), 3.03−3.20 (m, 2H), 3.38 (dt, J = 9.4, 5.8 Hz, 2H), 3.78 (q, J = 7.0 Hz, 1H), 4.64 (t, J = 5.3 Hz, 1H), 7.15 (dd, J = 8.2, 1.3 Hz, 1H), 7.34−7.37 (m, 1H), 7.45 (s, 1H), 7.47−7.49 (m, 1H), 7.96 (t, J = 5.5 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 8.16 (d, J = 2.0 Hz, 1H), 11.33 (s, 1H); MS (ES, m/z) $C_{17}H_{17}ClN_2O_2$ requires 316, found 317 $[M + H]^+$.

2-(6-Chloro-9*H*-carbazol-2-yl)-*N*-phenylpropanamide (4b). To a solution of **1** (126 mg, 0.46 mmol, 1 equiv) in pyridine (2 mL) was added CDI (149 mg, 0.92 mmol, 2 equiv) portionwise. The mixture was stirred for 2 h at room temperature, before addition of aniline (84 μL, 0.92 mmol, 2 equiv), and then the reaction was heated at 55 °C overnight. Pyridine was then evaporated and the residue taken up in EtOAc and washed with a saturated aqueous NH_4Cl solution (5 mL). After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (DCM/MeOH) to give amide **4b** (138 mg, 86%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.51 (d, J = 7.0 Hz, 3H), 4.00 (q, J = 6.9 Hz, 1H), 7.02 (t, J = 7.4 Hz, 1H), 7.23 (dd, J = 8.2, 1.3 Hz, 1H), 7.26−7.30 (m, 2H), 7.35−7.37 (m, 1H), 7.47−7.49 (m, 1H), 7.52 (s, 1H), 7.61 (d, J = 8.5 Hz, 2H), 8.10 (d, J = 8.1 Hz, 1H), 8.17 (d, J = 2.0 Hz, 1H), 10.07 (s, 1H), 11.36 (s, 1H); MS (ES, m/z) $C_{21}H_{17}ClN_2O$ requires 348, found 349 $[M + H]^+$.

General Procedure A. To a solution of **3** in MeCN (0.05 M solution) were successively added the alkyl halide (3 to 5 equiv) and Cs_2CO_3 (5 equiv). The mixture was heated under reflux overnight. After being cooled to room temperature, the mixture was filtered. EtOAc and H_2O were added to the filtrate. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to provide the alkylated product **5**.

*Methyl 2-(6-Chloro-9-methyl-9H-carbazol-2-yl)propanoate (**5a**).* Following general procedure A, alkylation of **3** (113 mg, 0.39 mmol) in the presence of MeI (0.12 mL, 1.96 mmol) and Cs_2CO_3 (756 mg, 1.96 mol) afforded the methylated product **5a** (118 mg, quantitative) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (d, J = 7.1 Hz, 3H), 3.69 (s, 3H), 3.83 (s, 3H), 3.93 (q, J = 7.1 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.33 (s, 1H), 7.30−7.42 (m, 1H), 7.97 (d, J = 8.1 Hz, 1H), 8.01 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{17}H_{16}ClNO_2$ requires 301, found 302 $[M + H]^+$.

*Methyl 2-(9-Benzyl-6-chloro-9H-carbazol-2-yl)propanoate (**5b**).* Following general procedure A, alkylation of **3** (103 mg, 0.39 mmol) in the presence of BnBr (0.13 mmol, 1,08 mmol) and Cs_2CO_3 (692 mg, 1.79 mmol) afforded the benzylated product **5b** (55 mg, 40%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (d, J = 7.3 Hz, 3H), 3.64 (s, 3H), 3.87 (q, J = 7.1 Hz, 1H), 5.49 (s, 2H), 7.11 (dd, J = 7.3, 2.1 Hz, 2H), 7.19−7.29 (m, 6H), 7.33−7.35 (m, 1H), 8.01 (d, J = 8.1 Hz, 1H), 8.04 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{23}H_{20}ClNO_2$ requires 377, found 378 $[M + H]^+$.

*Methyl 2-(6-Chloro-9-(4-cyanobenzyl)-9H-carbazol-2-yl)-propanoate (**5c**).* Following general procedure A, alkylation of **3** (112 mg, 0.39 mmol) in the presence of 4-cyanobenzyl bromide (380 mg, 1.94 mmol) and Cs_2CO_3 (785 mg, 1.94 mmol) afforded the 4-cyanobenzylated product **5c** (149 mg, 95%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.58 (d, J = 7.1 Hz, 3H), 3.66 (s, 3H), 3.89 (q, J = 7.1 Hz, 1H), 5.55 (s, 2H), 7.17 (d, J = 8.7 Hz, 1H), 7.20 (d, J = 8.5 Hz, 2H), 7.24−7.28 (m, 2H), 7.38 (dd, J = 8.6, 2.1 Hz, 1H), 7.58−7.60 (m, 2H), 8.04 (dd, J = 7.9, 0.8 Hz, 1H), 8.07 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{24}H_{19}ClN_2O_2$ requires 402, found 401 $[M - H]^-$.

*Methyl 2-(6-Chloro-9-(4-methoxybenzyl)-9H-carbazol-2-yl)-propanoate (**5e**).* Following general procedure A, alkylation of **3** (122 mg, 0.42 mmol) in the presence of 4-methoxybenzyl bromide (0.31 mL, 2.11 mmol) and Cs_2CO_3 (816 mg, 2.11 mmol) afforded the 4-methoxybenzylated product **5e** (99 mg, 55%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.57 (d, J = 7.2 Hz, 3H), 3.65 (s, 3H), 3.75 (s, 3H), 3.88 (q, J = 7.2 Hz, 1H), 5.42 (s, 2H), 6.79−6.81 (m, 2H), 7.06 (d, J = 8.7 Hz, 2H), 7.20 (dd, J = 8.1, 1.4 Hz, 1H), 7.25−7.27 (m, 1H), 7.29−7.31 (m, 1H), 7.33−7.35 (m, 1H), 8.00 (d, J = 8.1 Hz, 1H), 8.03 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{24}H_{22}ClNO_3$ requires 407, found 408 $[M + H]^+$.

General Procedure B. To a solution of the methyl ester in a 1:1:1 MeOH/THF/H_2O (0.03 M solution) was added LiOH (4 equiv). The mixture was stirred at room temperature overnight, and then the organic solvents were evaporated. The aqueous solution was then acidified to pH 1 with 6 M HCl. The precipitate formed was either (1) filtered, washed with H_2O, and dried under vacuum or (2) redissolved in EtOAc, dried over $MgSO_4$, and concentrated.

*2-(6-Chloro-9-methyl-9H-carbazol-2-yl)propanoic Acid (**6a**).* Following general procedure B, hydrolysis of ester **5a** (86 mg, 0.28 mmol) in the presence of LiOH (27 mg, 1.13 mmol) furnished acid **6a** (69

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mg, 85%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48 (d, J = 7.1 Hz, 3H), 3.86 (q, J = 7.1 Hz), 3.87 (s, 3H), 7.16 (dd, J = 8.2, 1.5 Hz, 1H), 7.45 (dd, J = 8.7, 2.1 Hz, 1H), 7.50 (d, J = 1.5 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{16}H_{14}ClNO_2$ requires 287, found 242 [M − H − CO_2]$^-$.

*2-(9-Benzyl-6-chloro-9H-carbazol-2-yl)propanoic Acid (**6b**).* Following general procedure B, hydrolysis of ester **5b** (40 mg, 0.10 mmol) in the presence of LiOH (10 mg, 0.42 mmol) furnished acid **6b** (27 mg, 73%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (d, J = 7.1 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 5.60−5.74 (m, 2H), 7.13−7.20 (m, 3H), 7.19−7.33 (m, 3H), 7.42 (dd, J = 8.7, 2.1 Hz, 1H), 7.58 (s, 1H), 7.62 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 8.1 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 12.31 (s, 1H); MS (ES, m/z) $C_{22}H_{18}ClNO_2$ requires 287, found 363 [M − H − CO_2]$^-$, 362 [M − H]$^-$.

*2-(6-Chloro-9-(4-cyanobenzyl)-9H-carbazol-2-yl)propanoic Acid (**6c**).* Following general procedure B, hydrolysis of ester **5c** (104 mg, 0.26 mmol) in the presence of LiOH (25 mg, 1.04 mmol), followed by purification by preparative TLC (DCM/MeOH, 95:5), furnished acid **6c** (52 mg, 67%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.43 (d, J = 7.1 Hz, 3H), 3.82 (q, J = 7.0 Hz, 1H), 5.80 (s, 2H), 7.19 (dd, J = 8.2, 1.4 Hz, 1H), 7.26 (d, J = 8.1 Hz, 2H), 7.43 (dd, J = 8.8, 2.1 Hz, 1H), 7.55 (d, J = 1.4 Hz, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.75−7.77 (m, 2H), 8.18 (d, J = 8.1 Hz, 1H), 8.28 (d, J = 2.1 Hz, 1H), 12.28 (s, 1H); MS (ES, m/z) $C_{23}H_{17}ClN_2O_2$ requires 388, found 343 [M − H − CO_2]$^-$.

*2-(6-Chloro-9-(4-chlorobenzyl)-9H-carbazol-2-yl)propanoic Acid (**6d**).* To a solution of **3** (144 mg, 0.5 mmol, 1 equiv) in MeCN (5 mL) were successively added 4-chlorobenzyl bromide (308 mg, 1.50 mmol, 3 equiv), Cs_2CO_3 (814 mg, 5 mmol, 5 equiv), and TBAI (92 mg, 0.25 mmol, 0.5 equiv). The mixture was stirred at room temperature overnight. The suspension was then passed through a silica plug and washed with EtOAc. After concentration in vacuo, the residue was dissolved in a 1:1:1 MeOH/THF/H_2O solution (3 mL). LiOH (24 mg, 1 mmol, 2 equiv) was added and the mixture was stirred at room temperature overnight. The solution was then acidified with 2 M HCl and extracted with EtOAc. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized in Et_2O to obtain acid **6d** (64 mg, 32%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (d, J = 7.1 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 5.67 (s, 2H), 7.15 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.2, 1.3 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.42 (dd, J = 8.7, 2.1 Hz, 1H), 7.56 (d, J = 1.3 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.25 (d, J = 2.0 Hz, 1H), 12.27 (s, 1H); MS (ES, m/z) $C_{22}H_{17}Cl_2NO_2$ requires 397, found 352 [M − H − CO_2]$^-$.

*2-(6-Chloro-9-(4-methoxybenzyl)-9H-carbazol-2-yl)propanoic Acid (**6e**).* Following general procedure B, hydrolysis of ester **5e** (55 mg, 0.13 mmol) in the presence of LiOH (12 mg, 0.52 mmol), followed by purification by preparative HPLC, furnished acid **6e** (18 mg, 35%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 3.68 (s, 3H), 3.84 (q, J = 7.1 Hz, 1H), 5.54−5.62 (m, 2H), 6.82−6.84 (m, 2H), 7.12−7.18 (m, 3H), 7.42 (dd, J = 8.7, 2.1 Hz, 1H), 7.61−7.65 (m, 2H), 8.15 (d, J = 8.1 Hz, 1H), 8.24 (d, J = 2.1 Hz, 1H), 12.29 (s, 1H); MS (ES, m/z) $C_{23}H_{20}ClNO_3$ requires 393, found 348 [M − H − CO_2]$^-$.

*2-(6-Chloro-9-(3-methoxybenzyl)-9H-carbazol-2-yl)propanoic Acid (**6f**).* To a solution of **3** (144 mg, 0.5 mmol, 1 equiv) in MeCN (5 mL) were successively added 3-methoxybenzyl bromide (0.21, 1.50 mmol, 3 equiv), Cs_2CO_3 (814 mg, 5 mmol, 5 equiv), and TBAI (92 mg, 0.25 mmol, 0.5 equiv). The mixture was stirred at room temperature overnight. The suspension was then passed through a silica plug and washed with EtOAc. After concentration in vacuo, the residue was dissolved in a 1:1:1 MeOH/THF/H_2O solution (3 mL). LiOH (24 mg, 1 mmol, 2 equiv) was added/ and the mixture was stirred at room temperature overnight. The solution was then acidified with 2 M HCl and extracted with EtOAc. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by preparative HPLC to obtain acid **6f** (42 mg, 21%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.44 (d, J = 7.1 Hz, 3H), 3.66 (s, 2H), 3.84 (q, J = 7.0 Hz, 1H), 5.62 (m, 3H), 6.66 (d, J = 7.6 Hz, 1H), 6.79 (m, 2H), 7.16 (m, 2H), 7.41 (dd, J = 8.7, 1.9 Hz, 1H), 7.59 (s, 1H), 7.62 (d, J = 8.8 Hz, 1H), 8.16 (d, J = 8.1 Hz, 1H), 8.24 (d, J = 1.8 Hz, 1H), 12.28 (s, 1H); MS (ES, m/z) $C_{23}H_{20}ClNO_3$ requires 393, found 348 [M − H − CO_2]$^-$.

*2-(6-Chloro-9-(2-methoxybenzyl)-9H-carbazol-2-yl)propanoic Acid (**6g**).* To a solution of **3** (144 mg, 0.5 mmol, 1 equiv) in MeCN (5 mL) were successively added 2-methoxybenzyl bromide (235 mg, 1.50 mmol, 3 equiv), Cs_2CO_3 (814 mg, 5 mmol, 5 equiv) and TBAI (92 mg, 0.25 mmol, 0.5 equiv). The mixture was stirred at room temperature overnight. The suspension was then passed through a silica plug and washed with EtOAc. After concentration in vacuo, the residue was dissolved in a 1:1:1 MeOH/THF/H_2O solution (3 mL). LiOH (24 mg, 1 mmol, 2 equiv) was added and the mixture was stirred at room temperature overnight. The solution was then acidified with 2 M HCl and extracted with EtOAc. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by preparative HPLC to obtain acid **6g** (46 mg, 23%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.42 (d, J = 7.1 Hz, 3H), 3.83 (q, J = 7.2 Hz, 1H), 3.86 (s, 3H), 5.55 (s, 2H), 6.65 (dd, J = 7.6, 1.7 Hz, 1H), 6.75 (td, J = 7.4, 1.0 Hz, 1H), 7.05 (dd, J = 8.3, 0.9 Hz, 1H), 7.16 (dd, J = 8.2, 1.4 Hz, 1H), 7.23 (ddd, J = 8.2, 7.4, 1.8 Hz, 1H), 7.40 (dd, J = 8.7, 2.1 Hz, 1H), 7.51 (d, J = 1.3 Hz, 1H), 7.56 (d, J = 8.7 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 8.24 (d, J = 2.0 Hz, 1H), 12.28 (s, 1H); MS (ES, m/z) $C_{23}H_{20}ClNO_3$ requires 393, found 348 [M − H − CO_2]$^-$.

*Methyl 2-(6-Chloro-9-(hexylsulfonyl)-9H-carbazol-2-yl)-propanoate (**7a**).* To a solution of **3** (105 mg, 0.36 mmol, 1 equiv) in THF (5 mL) were successively added 1-hexanesulfonyl chloride (0.17 mL, 1.09 mmol, 3 equiv), DMAP (133 mg, 1.09 mmol, 3 equiv), and Et_3N (0.15 mmol, 1.09 mmol, 3 equiv). The mixture was heated under reflux overnight, then filtered, and concentrated. The residue was purified by column chromatography (Cy/EtOAc) to give sulfonamide **7a** (55 mg, 35%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (t, J = 7.0 Hz, 3H), 1.11−1.30 (m, 6H), 1.58−1.68 (m, 5H), 3.19−3.23 (m, 2H), 3.71 (s, 3H), 3.94 (q, J = 7.2 Hz, 1H), 7.41 (dd, J = 8.1, 1.3 Hz, 1H), 7.45 (dd, J = 8.9, 2.1 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 2.0 Hz, 1H), 8.08−8.11 (m, 2H); MS (ES, m/z) $C_{22}H_{26}ClNO_4S$ requires 435, found 436 [M + H$^+$], 453 [M + NH_4^+].

*Methyl 2-(6-Chloro-9-((4-chlorophenyl)sulfonyl)-9H-carbazol-2-yl)propanoate (**7b**).* To a solution of **3** (110 mg, 0.38 mmol, 1 equiv) in THF (5 mL) were successively added 4-chlorobenzene-sulfonyl chloride (241 mg, 1.14 mmol, 3 equiv), DMAP (140 mg, 1.14 mml, 3 equiv), and Et_3N (0.16 mL, 1.14 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 3 h, then filtrated through a pad of Celite, washed with EtOAc, and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish sulfonamide **7b** (137 mg, 78%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (d, J = 7.2 Hz, 3H), 3.74 (s, 3H), 3.96 (q, J = 7.2 Hz, 1H), 7.32 (d, J = 8.7 Hz, 2H), 7.36 (dd, J = 8.1, 1.4 Hz, 1H), 7.47 (dd, J = 8.9, 2.1 Hz, 1H), 7.73 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.1 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H), 8.24−8.27 (m, 2H); MS (ES, m/z) $C_{22}H_{17}Cl_2NO_4S$ requires 461, found 460, 462 [M − H]$^-$.

*2-(6-Chloro-9-(hexylsulfonyl)-9H-carbazol-2-yl)propanoic Acid (**8a**).* Following general procedure B, hydrolysis of ester **7a** (55 mg, 0.13 mmol) in the presence of LiOH (12 mg, 0.50 mmol), followed by purification by column chromatography (Cy/EtOAc) and trituration with Et_2O, furnished acid **8a** (15 mg, 28%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 (t, J = 6.9 Hz, 3H), 0.99−1.08 (m, 4H), 1.15 (p, J = 7.3 Hz, 2H), 1.41−1.48 (m, 5H), 3.56 (t, J = 7.5 Hz, 2H), 3.91 (q, J = 7.0 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.58 (dd, J = 8.9, 2.2 Hz, 1H), 8.00 (s, 1H), 8.03 (d, J = 8.9 Hz, 1H), 8.24 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 2.1 Hz, 1H), 12.44 (s, 1H); MS (ES, m/z) $C_{21}H_{24}ClNO_4S$ requires 421, found 376 [M − H − CO_2]$^-$, 420 [M − H]$^-$.

*2-(6-Chloro-9-((4-chlorophenyl)sulfonyl)-9H-carbazol-2-yl)-propanoic Acid (**8b**).* Following general procedure B, hydrolysis of ester **7b** (133 mg, 0.29 mmol) in the presence of LiOH (27 mg, 1.15 mmol), followed by trituration with Et_2O, furnished acid **8b** (51 mg, 40%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.49 (d, J =

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7.0 Hz, 3H), 3.97 (q, J = 6.8 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.57−7.63 (m, 3H), 7.82 (d, J = 8.6 Hz, 2H), 8.14−8.17 (m, 2H), 8.25 (d, J = 8.9 Hz, 1H), 8.30 (s, 1H), 12.44 (s, 1H); MS (ES, m/z) $C_{21}H_{15}Cl_2NO_4S$ requires 447, found 402, 404 $[M - H - CO_2]^-$, 446, 448 $[M - H]^-$.

*Methyl 2-(6-Chloro-9-(hexylcarbamoyl)-9H-carbazol-2-yl)-propanoate (**9a**).* To a solution of **3** (100 mg, 0.35 mmol, 1 equiv) in THF (5 mL) were successively added hexyl isocyanate (0.10 mL, 0.70 mmol, 2 equiv), DMAP (85 mg, 0.70 mmol, 2 equiv), and Et_3N (0.15 mL, 1.05 mmol, 3 equiv). The mixture was heated under reflux for 60 h and then concentrated. The residue was taken up in EtOAc, and a saturated aqueous NH_4Cl solution (5 mL) was added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9a** (106 mg, 70%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, J = 6.6 Hz, 3H), 1.38 (dt, J = 7.6, 3.7 Hz, 4H), 1.46−1.50 (m, 2H), 1.60 (d, J = 7.1 Hz, 3H), 1.74 (p, J = 7.3 Hz, 2H), 3.56 (q, J = 6.7 Hz, 2H), 3.68 (s, 3H), 3.90 (q, J = 7.2 Hz, 1H), 5.67 (t, J = 5.6 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.40 (dd, J = 8.8, 2.0 Hz, 1H), 7.90−7.94 (m, 4H); MS (ES, m/z) $C_{23}H_{27}ClN_2O_3$ requires 414, found 415 $[M + H]^+$, 433 $[M + NH_4]^+$.

*Methyl 2-(6-Chloro-9-(methylcarbamoyl)carbazol-2-yl)-propanoate (**9b**).* To a solution of Boc_2O (0.392 mg, 1.79 mmol, 3 equiv) in MeCN (3 mL) were successively added DMAP (219 mg, 1.79 mmol, 3 equiv) and methylamine (2 M solution in THF, 0.89 mL, 1.79 mmol, 3 equiv). The mixture was stirred for 30 min at room temperature and then added to a solution of **3** (170 mg, 0.60 mmol, 1 equiv) in MeCN (2 mL). The mixture was heated in the microwave at 100 °C for 3 h. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9b** (139 mg, 68%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.48 (d, J = 7.1 Hz, 3H), 2.94 (d, J = 4.4 Hz, 3H), 3.60 (s, 3H), 3.99 (q, J = 7.1 Hz, 1H), 7.26 (dd, J = 1.3, 8.1 Hz, 1H), 7.50 (dd, J = 2.2, 8.8 Hz, 1H), 7.85 (s, 1H), 7.89 (d, J = 8.8 Hz, 1H), 8.18 (d, J = 8.1 Hz, 1H), 8.25 (q, J = 4.1 Hz, 1H), 8.28 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{18}H_{17}ClN_2O_3$ requires 344, found 345 $[M + H]^+$.

*Methyl 2-(9-(Butylcarbamoyl)-6-chloro-9H-carbazol-2-yl)-propanoate (**9c**).* To a solution of **3** (105 mg, 0.36 mmol, 1 equiv) in THF (5 mL) were successively added butyl isocyanate (0.12 mL, 1.09 mmol, 3 equiv), DMAP (133 mg, 1.09 mml, 3 equiv), and Et_3N (0.15 mL, 1.09 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 10 h. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9c** (97 mg, 80%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (t, J = 7.3 Hz, 3H), 1.51 (m, 2H), 1.60 (d, J = 7.2 Hz, 3H), 1.73 (m, 2H), 3.58 (td, J = 7.1, 5.5 Hz, 2H), 3.69 (s, 3H), 3.91 (q, J = 7.3 Hz, 1H), 5.66 (t, J = 5.7 Hz, 1H), 7.29 (m, 1H), 7.41 (dd, J = 8.9, 2.0 Hz, 1H), 7.93 (m, 4H); MS (ES, m/z) $C_{21}H_{23}ClN_2O_3$ requires 386, found 387 $[M + H]^+$, 404 $[M + NH_4]^+$.

*Methyl 2-(6-Chloro-9-(octylcarbamoyl)-9H-carbazol-2-yl)-propanoate (**9d**).* To a solution of **3** (106 mg, 0.37 mmol, 1 equiv) in THF (5 mL) were successively added octyl isocyanate (0.19 mL, 1.10 mmol, 3 equiv), DMAP (135 mg, 1.10 mml, 3 equiv), and Et_3N (0.15 mL, 1.10 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 10 h. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9d** (170 mg, 70%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86−0.91 (m, 3H), 1.26−1.47 (m, 10H), 1.60 (d, J = 7.2 Hz, 3H), 1.70−1.77 (m, 2H), 3.56 (td, J = 7.2, 5.5 Hz, 2H), 3.68 (s, 3H), 3.90 (q, J = 7.2 Hz, 1H), 5.68 (t, J = 5.4 Hz, 1H), 7.29 (dd, J = 8.0, 1.5 Hz, 1H), 7.40 (dd, J = 8.9, 2.1 Hz, 1H), 7.90−7.93 (m, 4H); MS (ES, m/z) $C_{25}H_{31}ClN_2O_3$ requires 442, found 443 $[M + H]^+$, 460 $[M + NH_4]^+$.

*Methyl 2-(6-Chloro-9-((4-chlorophenyl)carbamoyl)-9H-carbazol-2-yl)propanoate (**9e**).* To a solution of **3** (116 mg, 0.40 mmol, 1 equiv) in THF (5 mL) were successively added 4-chlorophenyl isocyanate (0.16 mL, 1.21 mmol, 3 equiv), DMAP (148 mg, 1.21 mml, 3 equiv), and Et_3N (0.17 mL, 1.21 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 10 h. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9e** (143 mg, 81%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.60 (d, J = 7.2 Hz, 3H), 3.69 (s, 3H), 3.91 (q, J = 7.2 Hz, 1H), 7.33 (dd, J = 8.0, 1.3 Hz, 1H), 7.40−7.44 (m, 3H), 7.54−7.59 (m, 3H), 7.92−7.98 (m, 4H); MS (ES, m/z) $C_{23}H_{18}Cl_2N_2O_3$ requires 440, found 441, 443 $[M + H]^+$, 458, 460 $[M + NH_4]^+$.

*Methyl 2-(6-Chloro-9-(hexylmethylcarbamoyl)carbazol-2-yl)-propanoate (**9f**).* To a solution of **3** (124 mg, 0.43 mmol, 1 equiv) in THF (5 mL) were successively added *N*-hexyl-*N*-methylcarbamoyl chloride (460 mg, 2.58 mmol, 6 equiv, freshly prepared from *N*-methylhexylamine (1.0 equiv) and triphosgene (0.33 equiv) in the presence of pyridine (1.0 equiv) in dry DCM), DMAP (158 mg, 1.29 mmol, 3 equiv), and Et_3N (0.18 mL, 1.27 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 3 h. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9f** (88 mg, 48%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (q, J = 6.7 Hz, 2H), 1.04 - 1.23 (m, 6H), 1.47 (dd, J = 1.3, 7.1 Hz, 3H), 1.59 (p, J = 7.2 Hz, 2H), 3.00 (d, J = 5.2 Hz, 3H), 3.35 - 3.48 (m, 2H), 3.60 (d, J = 1.4 Hz, 3H), 4.01 (q, J = 7.1 Hz, 1H), 5.75 (s, 1H), 7.26 (ddd, J = 1.5, 2.6, 8.2 Hz, 1H), 7.39 - 7.44 (m, 1H), 7.47 - 7.54 (m, 2H), 8.19 (d, J = 8.1 Hz, 1H), 8.30 (dd, J = 0.9, 1.8 Hz, 1H); MS (ES, m/z) $C_{24}H_{29}ClN_2O_3$ requires 428, found 429 $[M + H]^+$.

*Methyl 2-(6-Chloro-9-(cyclohexylcarbamoyl)carbazol-2-yl)-propanoate (**9g**).* To a solution of **3** (103 mg, 0.36 mmol, 1 equiv) in THF (5 mL) were successively added cyclohexyl isocyanate (0.10 mL, 0.70 mmol, 2 equiv), DMAP (85 mg, 0.70 mmol, 2 equiv), and Et_3N (0.15 mL, 1.05 mmol, 3 equiv). The mixture was heated under reflux for 60 h and then concentrated. EtOAc (10 mL) and a saturated aqueous NH_4Cl solution (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the urea **9g** (120 mg, 80%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18−1.57 (m, 5H), 1.60 (d, J = 7.2 Hz, 3H), 1.70 (dt, J = 3.7, 12.8 Hz, 1H), 1.82 (dt, J = 3.7, 13.0 Hz, 2H), 2.18 (d, J = 11.6 Hz, 2H), 3.69 (s, 3H), 3.91 (q, J = 7.2 Hz, 1H), 4.01 (tdt, J = 3.9, 7.8, 11.6 Hz, 1H), 5.54 (d, J = 7.5 Hz, 1H), 7.29 (dd, J = 1.4, 8.1 Hz, 1H), 7.41 (dd, J = 2.2, 8.8 Hz, 1H), 7.92 (d, J = 4.9 Hz, 1H), 7.93−7.96 (m, 3H); MS (ES, m/z) $C_{23}H_{25}ClN_2O_3$ requires 412, found 413 $[M + H]^+$.

*2-(6-Chloro-9-(hexylcarbamoyl)-9H-carbazol-2-yl)propanoic Acid (**10a**).* Following general procedure B, hydrolysis of ester **9a** (74 mg, 0.25 mmol) in the presence of LiOH (24 mg, 1 mmol), followed by trituration with EtOAc at 40 °C, furnished acid **10a** (28 mg, 39%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, J = 6.9 Hz, 3H), 1.33−1.36 (m, 4H), 1.37 (d, J = 7.2 Hz, 3H), 1.40−1.45 (m, 2H), 1.65 (p, J = 7.2 Hz, 2H), 3.34−3.40 (m, 2H), 3.37 (q, J = 7.2 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.45 (dd, J = 8.9, 2.2 Hz, 1H), 7.80 (s, 1H), 7.84 (d, J = 8.8 Hz, 1H), 8.05 (d, J = 8.1 Hz, 1H), 8.21 (d, J = 2.2 Hz, 1H), 8.34 (t, J = 5.5 Hz, 1H); MS (ES, m/z) $C_{22}H_{25}ClN_2O_3$ requires 400, found 401 $[M + H]^+$, 418 $[M + NH_4]^+$.

*2-(6-Chloro-9-(methylcarbamoyl)carbazol-2-yl)propanoic Acid (**10b**).* To a solution of ester **9b** (60 mg, 0.17 mmol) in THF (6 mL) was added a solution of 6 M HCl (4 mL). The solution was stirred for 3 days at room temperature. EtOAc and H_2O were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by trituration with Et_2O/pentane to furnish acid **10b** (43 mg, 75%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 2.94 (d, J =

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4.4 Hz, 3H), 3.86 (q, *J* = 7.0 Hz, 1H), 7.28 (d, *J* = 8.1 Hz, 1H), 7.49 (dd, *J* = 2.1, 8.8 Hz, 1H), 7.86 (s, 1H), 7.89 (d, *J* = 8.8 Hz, 1H), 8.17 (d, *J* = 8.1 Hz, 1H), 8.25 (q, *J* = 4.0 Hz, 2H), 8.27 (d, *J* = 2.1 Hz, 1H); MS (ES, *m*/*z*) $C_{17}H_{15}ClN_2O_3$ requires 330, found 331 $[M + H]^+$.

*2-(9-(Butylcarbamoyl)-6-chloro-9H-carbazol-2-yl)propanoic Acid (**10c**).* Following general procedure B, hydrolysis of ester **9b** (80 mg, 0.20 mmol) in the presence of LiOH (24 mg, 1 mmol), followed by purification by preparative HPLC, furnished acid **10c** (45 mg, 60%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.98 (t, *J* = 7.3 Hz, 3H), 1.47 (m, 5H), 1.66 (p, *J* = 7.2 Hz, 2H), 3.39 (dd, *J* = 13.3, 6.4 Hz, 2H), 3.86 (q, *J* = 7.0 Hz, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.51 (dd, *J* = 8.8, 2.0 Hz, 1H), 7.85−7.87 (m, 2H), 8.18 (d, *J* = 8.1 Hz, 1H), 8.28 (d, *J* = 1.9 Hz, 1H), 8.39 (t, *J* = 5.4 Hz, 1H), 12.34 (s, 1H); MS (ES, *m*/*z*) $C_{20}H_{21}ClN_2O_3$ requires 372, found 371 $[M - H]^-$.

*2-(6-Chloro-9-(octylcarbamoyl)-9H-carbazol-2-yl)propanoic Acid (**10d**).* Following general procedure B, hydrolysis of ester **9c** (120 mg, 0.27 mmol) in the presence of LiOH (26 mg, 1.08 mmol), followed by trituration with Et_2O, furnished acid **10d** (62 mg, 53%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (t, J = 6.7 Hz, 3H), 1.28−1.44 (m, 10H), 1.46 (d, *J* = 7.1 Hz, 3H), 1.66 (p, *J* = 7.0 Hz, 2H), 3.34−3.40 (m, 2H), 3.86 (q, *J* = 7.0 Hz, 1H), 7.29 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.50 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.84−7.87 (m, 2H), 8.18 (d, *J* = 8.0 Hz, 1H), 8.28 (d, *J* = 2.1 Hz, 1H), 8.40 (t, *J* = 5.5 Hz, 1H), 12.31 (s, 1H); MS (ES, *m*/*z*) $C_{24}H_{29}ClN_2O_3$ requires 428, found 429 $[M + H]^+$, 447 $[M + NH_4]^+$.

*2-(6-Chloro-9-((4-chlorophenyl)carbamoyl)-9H-carbazol-2-yl)-propanoic Acid (**10e**).* To a solution of ester **9d** (140 mg, 0.32 mmol) in THF (5 mL) was added a solution of 6 M HCl (5 mL). The solution was stirred for 5 days at room temperature. EtOAc (10 mL) and H_2O (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was triturated with Et_2O/pentane to furnish acid **10e** (105 mg, 80%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (d, *J* = 7.1 Hz, 3H), 3.90 (q, *J* = 7.0 Hz, 1H), 7.35 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.50 (d, *J* = 8.9 Hz, 2H), 7.55 (m, 1H), 7.73 (d, *J* = 8.9 Hz, 2H), 7.88 (s, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 8.24 (d, *J* = 8.1 Hz, 1H), 8.34 (s, 1H), 10.74 (s, 1H); MS (ES, *m*/*z*) $C_{22}H_{16}Cl_2N_2O_3$ requires 426, found 427, 429 $[M + H]^+$, 444, 446 $[M + NH_4]^+$.

*2-(6-Chloro-9-(hexylmethylcarbamoyl)carbazol-2-yl)propanoic Acid (**10f**).* Following general procedure B, hydrolysis of ester **9f** (60 mg, 0.14 mmol) in the presence of LiOH (8 mg, 0.35 mmol), followed by trituration with DCM/MeOH, furnished acid **10f** (45 mg, 77%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.71 - 0.79 (m, 2H), 1.16 (m, 6H), 1.44 (dd, *J* = 2.8, 7.1 Hz, 3H), 1.60 (t, *J* = 7.1 Hz, 2H), 3.00 (d, *J* = 1.7 Hz, 3H), 3.41 (h, *J* = 7.2 Hz, 2H), 3.87 (q, *J* = 7.1 Hz, 1H), 5.75 (s, 1H), 7.28 (dt, *J* = 1.6, 8.2 Hz, 1H), 7.43 (d, *J* = 1.3 Hz, 1H), 7.46 - 7.53 (m, 2H), 8.18 (d, *J* = 8.1 Hz, 1H), 8.29 (dd, *J* = 0.9, 1.8 Hz, 1H), 12.36 (s, 1H); MS (ES, *m*/*z*) $C_{23}H_{27}ClN_2O_3$ requires 414, found 415 $[M + H]^+$.

*2-(6-Chloro-9-(cyclohexylcarbamoyl)carbazol-2-yl)propanoic Acid (**10g**).* Following general procedure B, hydrolysis of ester **9g** (60 mg, 0.14 mmol) in the presence of LiOH (8 mg, 0.35 mmol), followed by trituration with Et_2O, furnished acid **10g** (51 mg, 89%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17−1.31 (m, 2H), 1.43 (dd, *J* = 9.1, 16.1 Hz, 6H), 1.63 (d, *J* = 12.8 Hz, 1H), 1.72 - 1.84 (m, 2H), 2.02 (d, *J* = 9.6 Hz, 2H), 3.68−3.81 (m, 1H), 3.85 (q, *J* = 7.1 Hz, 1H), 7.27 (dd, *J* = 1.3, 8.1 Hz, 1H), 7.49 (dd, *J* = 2.2, 8.8 Hz, 1H), 7.79−7.87 (m, 2H), 8.16 (d, *J* = 8.1 Hz, 1H), 8.27 (d, *J* = 2.1 Hz, 1H), 8.35 (d, *J* = 7.5 Hz, 1H), 12.34 (s, 1H); MS (ES, *m*/*z*) $C_{22}H_{23}ClN_2O_3$ requires 398, found 399 $[M + H]^+$.

*Hexyl 6-Chloro-2-(1-methoxy-1-oxoprop-2-yl)-9H-carbazole-9-carboxylate (**11**).* To a solution of **3** (140 mg, 0.49 mmol, 1 equiv) in THF (5 mL) were successively added hexyl chloroformate (freshly prepared, 215 mg, 1.47 mmol, 3 equiv), DMAP (179 mg, 1.47 mml, 3 equiv), and Et_3N (0.20 mL, 1.47 mmol, 3 equiv). The mixture was heated in the microwave at 100 °C for 3 h. The reaction mixture was then filtrated and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish the carbamate **11** (173 mg, 85%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.33−1.37 (m, 4H), 1.48−1.53 (m, 5H), 1.84−1.91 (m, 2H), 3.61 (s, 3H), 4.02 (q, *J* = 7.0 Hz, 1H), 4.52 (t, *J* = 6.5 Hz, 2H), 7.37 (dd, *J* = 8.0, 1.5 Hz, 1H), 7.56 (dd, *J* = 8.9, 2.2 Hz, 1H), 8.20−8.25 (m, 3H), 8.31 (d, *J* = 2.1 Hz, 1H); MS (ES, *m*/*z*) $C_{23}H_{26}ClNO_4$ requires 415, found 433 $[M + NH_4]^+$.

*2-(6-Chloro-9-((hexyloxy)carbonyl)-9H-carbazol-2-yl)propanoic Acid (**12**).* To a solution of ester **11** (87 mg, 0.21 mmol) in THF (2.5 mL) was added a solution of 6 M HCl (2.5 mL). The solution was stirred for 5 days at room temperature. EtOAc (10 mL) and H_2O (5 mL) were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to furnish acid **12** (46 mg, 55%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.31−139 (m, 4H), 1.46 (d, *J* = 7.1 Hz, 3H), 1.49−1.54 (m, 2H), 1.84−1.91 (m, 2H), 3.88 (q, *J* = 7.1 Hz, 1H), 4.52 (t, *J* = 6.5 Hz, 2H), 7.38 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.55 (dd, *J* = 8.9, 2.2 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 8.22−8.24 (m, 2H), 8.30 (d, *J* = 2.2 Hz, 1H), 12.38 (s, 1H); MS (ES, *m*/*z*) $C_{22}H_{24}ClNO_4$ requires 401, found 400 $[M - H]^-$, 356 $[M - H - CO_2]^-$.

*Benzyl 2-(6-Chloro-9H-carbazol-2-yl)propanoate (**13**).*[43] To a solution of **1** (773 mg, 2.82 mmol, 1 equiv) in DMF (10 mL) was added K_2CO_3 (1.17 g, 8.47 mmol, 3 equiv). The mixture was stirred at room temperature for 30 min, and then BnBr (0.37 mL, 3.10 mmol, 1.1 equiv) was added. After 3 h at room temperature, EtOAc was added (20 mL), and the mixture was washed with a saturated aqueous NH_4Cl solution (5 mL) and with H_2O (5 mL). The organic phase was then dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to yield the corresponding benzyl ester **13** (860 mg, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (d, *J* = 7.2 Hz, 3H), 3.94 (q, *J* = 7.1 Hz, 1H), 5.13 (q, *J* = 12.5 Hz, 2H), 7.19 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.23−7.25 (m, 2H), 7.28−7.36 (m, 6H), 7.94 (d, *J* = 8.1 Hz, 1H), 7.99 (d, *J* = 1.8 Hz, 1H), 8.03 (s, 1H); MS (ES, *m*/*z*) $C_{22}H_{18}ClNO_2$ requires 363, found 362 $[M - H]^-$.

General Procedure C. To a solution of **13** (or **3** in the case of **14j**) in MeCN (5 mL) were successively added the acyl chloride (3 equiv, either obtained from a commercial source or freshly prepared from the corresponding acid), DMAP (3 equiv), and Et_3N (3 equiv). The mixture was stirred at room temperature for 2 h, and then a saturated aqueous NH_4Cl solution and H_2O were added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc).

*Benzyl 2-(9-Acetyl-6-chloro-9H-carbazol-2-yl)propanoate (**14a**).* Following general procedure C, acylation of **13** (185 mg, 0.51 mmol) in the presence of AcCl (0.11 mL, 1.52 mmol), DMAP (186 mg, 1.52 mmol), and Et_3N (0.21 mL, 1.52 mmol) furnished the acetyl derivative **14a** (200 mg, 97%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (d, *J* = 7.2 Hz, 3H), 2.82 (s, 3H), 3.98 (q, *J* = 7.2 Hz, 1H), 5.12−5.19 (m, 2H), 7.25−7.32 (m, 5H), 7.37 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.44 (dd, *J* = 8.9, 2.2 Hz, 1H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 2.1 Hz, 1H), 8.08 (s, 1H), 8.24 (d, *J* = 8.9 Hz, 1H); MS (ES, *m*/*z*) $C_{24}H_{20}ClNO_3$ requires 405, found 406 $[M + H^+]$, 423 $[M + NH_4^+]$.

*Benzyl 2-(9-Benzoyl-6-chloro-9H-carbazol-2-yl)propanoate (**14b**).* Following general procedure C, acylation of **13** (105 mg, 0.29 mmol) in the presence of benzoyl chloride (0.10 mL, 0.87 mmol), DMAP (106 mg, 0.87 mmol), and Et_3N (0.12 mL, 0.87 mmol) furnished the benzoyl derivative **14b** (123 mg, 91%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (d, *J* = 7.2 Hz, 3H), 3.81 (q, *J* = 7.1 Hz, 1H), 5.06−5.14 (m, 2H), 7.23−7.26 (m, 2H), 7.29−7.34 (m, 5H), 7.38 (s, 1H), 7.50−7.54 (m, 3H), 7.65−7.70 (m, 3H), 7.91 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 2.1 Hz, 1H); MS (ES, *m*/*z*) $C_{29}H_{22}ClNO_3$ requires 467, found 468 $[M + H^+]$, 485 $[M + NH_4^+]$.

*Benzyl 2-(6-Chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)-propanoate (**14c**).* Following general procedure C, acylation of **13** (98 mg, 0.27 mmol) in the presence of 4-chlorobenzoyl chloride (0.10 mL, 0.80 mmol), DMAP (98 mg, 0.80 mmol), and Et_3N (0.11 mL, 0.80 mmol) furnished the 4-chlorobenzoyl derivative **14c** (119 mg, 88%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (d, *J* = 7.2 Hz, 3H), 3.81 (q, *J* = 7.2 Hz, 1H), 5.06−5.13 (m, 2H), 7.21−7.24 (m, 2H), 7.28−7.33 (m, 5H), 7.40 (s, 1H), 7.47 (dd, *J* = 8.6, 5.6 Hz, 3H),

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7.62 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{29}H_{21}Cl_2NO_3$ requires 501, found 502 [M + H^+], 519 [M + NH_4^+].

*Benzyl 2-(6-Chloro-9-(4-fluorobenzoyl)-9H-carbazol-2-yl)-propanoate (**14d**).* Following general procedure C, acylation of **13** (98 mg, 0.27 mmol) in the presence of 4-fluorobenzoyl chloride (0.10 mL, 0.80 mmol), DMAP (98 mg, 0.80 mmol), and Et_3N (0.11 mL, 0.80 mmol) furnished the 4-fluorobenzoyl derivative **14d** (104 mg, 88%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (d, J = 7.2 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 5.07−5.14 (m, 2H), 7.18 (t, J = 8.6 Hz, 2H), 7.23−7.25 (m, 2H), 7.31−7.35 (m, 5H), 7.40 (s, 1H), 7.52 (d, J = 8.9 Hz, 1H), 7.72 (dd, J = 8.8, 5.3 Hz, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.97 (d, J = 1.9 Hz, 1H); MS (ES, m/z) $C_{29}H_{21}ClFNO_3$ requires 485, found 486 [M + H^+].

*Benzyl 2-(6-Chloro-9-(4-methoxybenzoyl)-9H-carbazol-2-yl)-propanoate (**14e**).* Following general procedure C, acylation of **13** (137 mg, 0.37 mmol) in the presence of 4-methoxybenzoyl chloride (0.15 mL, 1.12 mmol), DMAP (138 mg, 1.12 mmol), and Et_3N (0.16 mL, 1.12 mmol) furnished the 4-methoxybenzoyl derivative **14e** (156 mg, 85%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (d, J = 7.2 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 3.90 (s, 3H), 5.05−5.13 (m, 2H), 6.96 (d, J = 8.8 Hz, 2H), 7.21−7.23 (m, 2H), 7.26−7.31 (m, 5H), 7.46−7.49 (m, 2H), 7.67 (d, J = 4.9 Hz, 2H), 7.90 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{30}H_{24}ClNO_4$ requires 497, found 498 [M + H]$^+$.

*Benzyl 2-(6-Chloro-9-(3-chlorobenzoyl)-9H-carbazol-2-yl)-propanoate (**14f**).* Following general procedure C, acylation of **13** (112 mg, 0.32 mmol) in the presence of 3-chlorobenzoyl chloride (0.12 mL, 0.96 mmol), DMAP (117 mg, 0.96 mmol), and Et_3N (0.13 mL, 0.96 mmol) furnished the 3-chlorobenzoyl derivative **14f** (124 mg, 77%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (d, J = 7.2 Hz, 3H), 3.80 (q, J = 7.1 Hz, 1H), 5.04−5.13 (m, 2H), 7.21−7.23 (m, 2H), 7.28−7.34 (m, 6H), 7.42 (m, 1H), 7.53 (d, J = 8.4 Hz, 2H), 7.61−7.63 (m, 1H), 7.67 (t, J = 1.7 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{29}H_{21}Cl_2NO_3$ requires 501, found 502 [M + H^+], 519 [M + NH_4^+].

*Benzyl 2-(6-Chloro-9-(2-chlorobenzoyl)-9H-carbazol-2-yl)-propanoate (**14g**).* Following general procedure C, acylation of **13** (105 mg, 0.29 mmol) in the presence of 2-chlorobenzoyl chloride (0.11 mL, 0.86 mmol), DMAP (105 mg, 0.86 mmol), and Et_3N (0.12 mL, 0.86 mmol) furnished the 2-chlorobenzoyl derivative **14g** (129 mg, 88%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (d, J = 7.0 Hz, 3H), 3.77 (q, J = 7.1 Hz, 1H), 5.04−5.12 (m, 2H), 7.22−7.33 (m, 8H), 7.43−7.57 (m, 5H), 7.86 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 2.1 Hz, 1H); MS (ES, m/z) $C_{29}H_{21}Cl_2NO_3$ requires 501, found 502 [M + H^+], 519 [M + NH_4^+].

*Benzyl 2-(6-Chloro-9-(3,4-dichlorobenzoyl)-9H-carbazol-2-yl)-propanoate (**14h**).* Following general procedure C, acylation of **13** (120 mg, 0.33 mmol) with 3,4-dichlorobenzoyl chloride (freshly prepared from 3,4-dichlorobenzoic acid, 1 mmol), DMAP (122 mg, 1 mmol), and Et_3N (0.14 mL, 1 mmol) furnished the 3,4-dichlorobenzoyl derivative **14h** (157 mg, 89%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (d, J = 7.2 Hz, 3H), 3.83 (q, J = 7.1 Hz, 1H), 5.05−5.14 (m, 2H), 7.22−7.25 (m, 2H), 7.29−7.34 (m, 5H), 7.40 (s, 1H), 7.46−7.50 (m, 2H), 7.54−7.56 (m, 1H), 7.79 (d, J = 1.9 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{29}H_{20}Cl_3NO_3$ requires 535, found 536, 538 [M + H^+], 553, 555 [M + NH_4^+].

*Benzyl 2-(6-Chloro-9-(oxazol-4-ylcarbonyl)-9H-carbazol-2-yl)-propanoate (**14i**).* Following general procedure C, acylation of **13** (144 mg, 0.39 mmol) with oxazol-4-ylcarbonyl chloride (freshly prepared from oxazole-4-carboxylic acid, 1.20 mmol), DMAP (145 mg, 1.20 mmol), and Et_3N (0.16 mL, 1.20 mmol) furnished the oxazol-4-ylcarbonyl derivative **14i** (135 mg, 76%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.53 (d, J = 7.2 Hz, 3H), 3.86 (q, J = 7.1 Hz, 1H), 5.06−5.14 (m, 2H), 7.23−7.25 (m, 2H), 7.28−7.35 (m, 5H), 7.58 (d, J = 0.9 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.93 (m, 2H), 8.33 (s, 1H); MS (ES, m/z) $C_{26}H_{19}ClN_2O_4$ requires 458, found 459 [M + H^+], 476 [M + NH_4^+].

*tert-Butyl 4-(2-(1-(Benzyloxy)-1-oxoprop-2-yl)-6-chloro-9H-carbazol-9-ylcarbonyl)-1H-imidazole-1-carboxylate (**14j**).* To a suspension of imidazole-4-carboxylic acid (143 mg, 1.28 mmol, 1 equiv) in DMF (1.5 mL) were successively added Et_3N (0.35 mL, 2.56 mmol, 2 equiv) and a solution of Boc_2O (307 mg, 1.08 mmol, 1.1 equiv) in DMF (1.5 mL). After 4 h at room temperature, the solution was concentrated. The residue was taken up in EtOAc and washed with H_2O. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was suspended in DCM (5 mL), and oxalyl chloride (0.26 mL, 3.07 mmol, 2.4 equiv) followed by DMF (0.05 mL) was added. The solution was stirred for 2 h at room temperature and then concentrated in vacuo to provide the crude (1-((*tert*-butyloxy)carbonyl)imidazol-4-yl)carbonyl chloride as a yellow oil. To a solution of **13** (120 mg, 0.33 mmol, 1 equiv) in MeCN (3 mL) were successively added the crude acyl chloride described previously (1.28 mmol, 3.9 equiv) in MeCN (2 mL), DMAP (156 mg, 1.28 mmol, 3.9 equiv), and Et_3N (0.18 mL, 1.28 mmol, 3.9 equiv). The mixture was stirred at room temperature for 2 h,and then a saturated aqueous NH_4Cl solution and H_2O were added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography (Cy/EtOAc) to give the *N*-((*tert*-butyloxy)carbonyl)carbonylimidazole compound **14j** (46 mg, 25%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.56 (d, J = 7.2 Hz, 3H), 1.70 (s, 9H), 3.89 (q, J = 7.1 Hz, 1H), 5.06−5.17 (m, 2H), 7.24−7.34 (m, 7H), 7.65 (d, J = 8.8 Hz, 1H), 7.68 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 1.9 Hz, 1H), 8.13 (d, J = 1.2 Hz, 1H), 8.17 (d, J = 1.2 Hz, 1H); MS (ES, m/z) $C_{31}H_{28}ClN_3O_5$ requires 557, found 558 [M + H]$^+$.

*Methyl 2-(6-Chloro-9-(thiazol-4-ylcarbonyl)-9H-carbazol-2-yl)-propanoate (**14k**).* Following general procedure C, acylation of **3** (117 mg, 0.41 mmol) with thiazol-4-ylcarbonyl chloride (freshly prepared from thiazole-4-carboxylic acid, 1.22 mmol), DMAP (149 mg, 1.22 mmol), and Et_3N (0.17 mL, 1.22 mmol) furnished the thiazol-4-ylcarbonyl derivative **14k** (115 mg, 71%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (d, J = 7.2 Hz, 3H), 3.67 (s, 3H), 3.80 (q, J = 7.1 Hz, 1H), 7.27 − 7.35 (m, 3H), 7.48 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 2.1 Hz, 1H), 8.27 (d, J = 2.0 Hz, 1H), 8.94 (d, J = 2.0 Hz, 1H); MS (ES, m/z) $C_{20}H_{15}ClN_2O_3S$ requires 398, found 399 [M + H]$^+$.

*2-(9-Acetyl-6-chloro-9H-carbazol-2-yl)propanoic Acid (**15a**).* A solution of benzyl ester **14a** (100 mg, 0.25 mmol) in THF (30 mL) was hydrogenated with the ThalesNano H-Cube using a 1% Pd/C catalyst, at a 1 mL/min flow rate and room temperature and in a H_2 atmosphere (1 atm) (full H_2 mode), in a closed system for 5 h. The solvent was then removed in vacuo, and the product was crystallized from DCM/pentane to give acid **15a** (15 mg, 19%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.46 (d, J = 7.1 Hz, 3H), 2.88 (s, 3H), 3.91 (q, J = 7.1 Hz, 1H), 7.37−7.39 (m, 1H), 7.53 (dd, J = 8.9, 2.2 Hz, 1H), 8.20−8.25 (m, 3H), 8.31 (d, J = 2.2 Hz, 1H), 12.39 (s, 1H); MS (ES, m/z) $C_{17}H_{14}ClNO_3$ requires 315, found 314 [M − H]$^-$, 270 [M − H − CO_2]$^-$.

General Procedure D. A solution of benzyl ester **14** in THF (5 mM solution) was hydrogenated with the ThalesNano H-Cube using a 1% or 5% Pd/C catalyst, at a 1 mL/min flow rate and room temperature and in a H_2 atmosphere (1 atm) (full H_2 mode). The solvent was then removed in vacuo, and the crude oil was purified by preparative HPLC to yield the corresponding acid **15**.

*2-(9-Benzoyl-6-chloro-9H-carbazol-2-yl)propanoic Acid (**15b**).* Following general procedure D (5% Pd/C catalyst), ester **14b** (120 mg, 0.25 mmol) was hydrogenated to yield acid **15b** (29 mg, 31%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29 (d, J = 7.1 Hz, 3H), 3.72 (q, J = 7.1 Hz, 1H), 7.34−7.37 (m, 3H), 7.39−7.42 (m, 1H), 7.60−7.64 (m, 2H), 7.72−7.78 (m, 3H), 8.22 (d, J = 8.4 Hz, 1H), 8.34 (d, J = 2.0 Hz, 1H), 12.31 (s, 1H); MS (ES, m/z) $C_{22}H_{16}ClNO_3$ requires 377, found 378 [M + H^+], 395 [M + NH_4^+].

*2-(6-Chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15c**).* Following general procedure D (5% Pd/C catalyst), ester **14c** (119 mg, 0.23 mmol) was hydrogenated to yield acid **15c** (40 mg, 42%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (d, J = 7.1 Hz, 3H), 3.76 (q, J = 7.0 Hz, 1H), 7.33−7.40 (m, 2H), 7.39−7.47

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(m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 2.1 Hz, 1H), 12.35 (s, 1H); MS (ES, m/z) $C_{22}H_{15}ClNO_3$ requires 411, found 366 $[M - H - CO_2]^-$.

*2-(6-Chloro-9-(4-fluorobenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15d**).* Following general procedure D (5% Pd/C catalyst), ester **14d** (104 mg, 0.21 mmol) was hydrogenated to yield acid **15d** (35 mg, 42%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (d, J = 7.1 Hz, 3H), 3.75 (q, J = 7.0 Hz, 1H), 7.35−7.39 (m, 3H), 7.42−7.47 (m, 3H), 7.83 (dd, J = 8.6, 5.4 Hz, 2H), 8.22 (d, J = 8.6 Hz, 1H), 8.34 (d, J = 2.0 Hz, 1H), 12.33 (s, 1H); MS (ES, m/z) $C_{22}H_{15}ClFNO_3$ requires 395, found 396 $[M + H^+]$, 413 $[M + NH_4^+]$.

*2-(6-Chloro-9-(4-methoxybenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15e**).* Following general procedure D (1% Pd/C catalyst), ester **14e** (0.156 g, 0.31 mmol) was hydrogenated to yield acid **15e** (28 mg, 22%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.33 (d, J = 7.1 Hz, 3H), 3.76 (q, J = 7.0 Hz, 1H), 3.90 (s, 3H), 7.13 (d, J = 8.8 Hz, 2H), 7.33 (t, J = 8.9 Hz, 2H), 7.39−7.42 (m, 1H), 7.47 (s, 1H), 7.71 (d, J = 8.8 Hz, 2H), 8.21 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 2.1 Hz, 1H), 12.32 (s, 1H); MS (ES, m/z) $C_{23}H_{18}ClNO_4$ requires 407, found 408 $[M + H]^+$.

*2-(6-Chloro-9-(3-chlorobenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15f**).* Following general procedure D (1% Pd/C catalyst), ester **14f** (197 mg, 0.39 mmol) was hydrogenated to yield acid **15f** (25 mg, 15%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (d, J = 7.1 Hz, 3H), 3.74 (q, J = 7.1 Hz, 1H), 7.33−7.37 (m, 2H), 7.40−7.45 (m, 2H), 7.62−7.66 (m, 1H), 7.68−7.70 (m, 1H), 7.81−7.85 (m, 2H), 8.21 (d, J = 8.0 Hz, 1H), 8.34 (s, 1H), 12.33 (s, 1H); MS (ES, m/z) $C_{22}H_{15}Cl_2NO_3$requires 411, found 412 $[M + H^+]$, 434 $[M + Na^+]$.

*2-(6-Chloro-9-(2-chlorobenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15g**).* Following general procedure D (1% Pd/C catalyst), ester **14g** (125 mg, 0.25 mmol) was hydrogenated to yield acid **15g** (22 mg, 21%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26 (d, J = 7.1 Hz, 3H), 3.71 (q, J = 6.9 Hz, 1H), 7.23−7.25 (m, 1H), 7.38 (d, J = 7.7 Hz, 1H), 7.44 (d, J = 6.6 Hz, 2H), 7.63−7.67 (m, 1H), 7.71−7.77 (m, 2H), 7.84 (d, J = 7.2 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.35 (s, 1H), 12.34 (s, 1H); MS (ES, m/z) $C_{22}H_{15}Cl_2NO_3$ requires 411, found 412 $[M + H^+]$, 429 $[M + NH_4]^+$.

*2-(6-Chloro-9-(3,4-dichlorobenzoyl)-9H-carbazol-2-yl)propanoic Acid (**15h**).* Following general procedure D (5% Pd/C catalyst), ester **14h** (157 mg, 0.29 mmol) was hydrogenated to yield acid **15h** (35 mg, 28%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (d, J = 7.1 Hz, 3H), 3.78 (q, J = 7.0 Hz, 1H), 7.37 - 7.44 (m, 4H), 7.74 (dd, J = 8.3, 2.0 Hz, 1H), 7.89 (d, J = 8.3 Hz, 1H), 8.08 (d, J = 1.9 Hz, 1H), 8.22 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 1.7 Hz, 1H), 12.35 (s, 1H); MS (ES, m/z) $C_{22}H_{14}Cl_3NO_3$ requires 445, found 400, 402 $[M - H - CO_2]^-$.

*2-(6-Chloro-9-(oxazol-4-ylcarbonyl)-9H-carbazol-2-yl)propanoic Acid (**15i**).* Following general procedure D (1% Pd/C catalyst), ester **14i** (130 mg, 0.28 mmol) was hydrogenated to yield acid **15i** (21 mg, 20%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (d, J = 7.1 Hz, 3H), 3.82 (q, J = 7.0 Hz, 1H), 7.37 (dd, J = 8.1, 1.1 Hz, 1H), 7.44 (dd, J = 8.9, 2.2 Hz, 1H), 7.58 - 7.63 (m, 2H), 8.21 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 2.1 Hz, 1H), 8.69 (s, 1H), 9.06 (s, 1H), 12.38 (s, 1H); MS (ES, m/z) $C_{19}H_{13}ClN_2O_4$ requires 368, found 323 $[M - H - CO_2]^-$.

*2-(6-Chloro-9-(1H-imidazol-4-ylcarbonyl)-9H-carbazol-2-yl)-propanoic Acid Hydrochloride (**15j**).* To a solution of **14j** (84 mg, 0.15 mmol) in acetone (2 mL) was added 2 M HCl (2 mL). The mixture was stirred at room temperature overnight. After evaporation of the solvents, the residue was then dissolved in THF (40 mL), and the solution was hydrogenated using the ThalesNano H-Cube using a 5% Pd/C catalyst, at a 1 mL/min flow rate and room temperature and in a H_2 atmosphere (1 atm) (full H_2 mode). The solvent was then removed in vacuo, and the crude oil was purified by preparative HPLC. After evaporation of the solvents, the residue was dissolved in a 1:1 mixture of MeCN/H_2O and 0.5 mL of concentrated HCl. The solution was lyophilized overnight to afford the acid **15j** (11 mg, 18%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.39 (d, J = 7.1 Hz, 3H), 3.81 (q, J = 7.0 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.42 (dd, J = 8.9, 2.0 Hz, 1H), 7.55 (d, J = 8.9 Hz, 1H), 7.60 (s, 1H), 8.16 (s, 1H), 8.20 (d, J = 8.1 Hz, 1H), 8.27 (s, 1H), 8.30 (d, J = 1.9 Hz, 1H); MS (ES, m/z) $C_{19}H_{14}ClN_3O_3$ requires 367, found 322 $[M - H - CO_2]^-$, 366 $[M - H]^-$.

*2-(6-Chloro-9-(thiazol-4-ylcarbonyl)-9H-carbazol-2-yl)propanoic Acid (**15k**).* To a solution of ester **14k** (115 mg, 0.29 mmol) in THF (5 mL) was added a solution of 6 M HCl (5 mL). The solution was stirred for 5 days at room temperature. EtOAc and H_2O were then added. After separation, the organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by preparative HPLC to furnish acid **15k** (18 mg, 16%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (d, J = 7.1 Hz, 3H), 3.76 (q, J = 7.1 Hz, 1H), 7.20 (s, 1H), 7.34 - 7.41 (m, 2H), 7.45 (dd, J = 8.9, 2.2 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.34 (s, 1H), 8.74 (s, 1H), 9.29 (s, 1H), 12.35 (s, 1H); MS (ES, m/z) $C_{19}H_{13}ClN_2O_3S$ requires 384, found 339 $[M - H - CO_2]^-$.

Separation of Enantiomers. Compounds **1**, **15c**, and **15i** were subjected to enantiomeric separation by chiral HPLC. For **1**, the absolute configuration of each enantiomer was assigned by measuring the optical rotation and comparing the values to the data described in the literature.[42] The absolute configuration of each enantiomer of compounds **15c** and **15i** was assigned by chemical correlation. To this purpose, each enantiomer of **15c** and **15i** was separately hydrolyzed with 6 M HCl in THF for 3 days at 40 °C to give (*S*)- and (*R*)-**1**. The absolute configuration of each enantiomer was assigned by comparison of the chiral HPLC chromatogram with that of samples of (+)-(*S*)- and (-)-(*R*)-**1**. The results were consistent with the formation of a single enantiomer of **1** in each experiment.

*Data for (2S)-2-(6-chloro-9H-carbazol-2-yl)propanoic acid [(+)-**1**]:* $[\alpha]_D$ = +58.32 (c = 0.1, MeOH); ee > 99.5% (detector UV 240 nm); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 3.84 (q, J = 7.0 Hz, 1H), 7.12 (dd, J = 8.1, 1.3 Hz, 1H), 7.37 (dd, J = 8.6, 2.1 Hz, 1H), 7.41 (s, 1H), 7.49 (d, J = 8.6 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 11.36 (s, 1H), 12.32 (s, 1H); MS (ES, m/z) $C_{15}H_{12}ClNO_2$ requires 273, found 272 $[M - H]^-$; retention time on analytical chiral HPLC, 8.295 min.

*Data for (2R)-2-(6-chloro-9H-carbazol-2-yl)propanoic acid [(−)-**1**]:* $[\alpha]_D$ = −58.25 (c = 0.1, MeOH); ee > 99.5% (detector UV 240 nm); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 3.84 (q, J = 7.0 Hz, 1H), 7.12 (dd, J = 8.1, 1.3 Hz, 1H), 7.37 (dd, J = 8.6, 2.1 Hz, 1H), 7.41 (s, 1H), 7.49 (d, J = 8.6 Hz, 1H), 8.09 (d, J = 8.1 Hz, 1H), 8.18 (d, J = 2.0 Hz, 1H), 11.36 (s, 1H), 12.29 (s, 1H); MS (ES, m/z) $C_{15}H_{12}ClNO_2$ requires 273, found 272 $[M - H]^-$; retention time on analytical chiral HPLC, 10.173 min.

*Data for (2S)-(6-chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)-propanoic acid [(+)-**15c**]:* $[\alpha]_D$ = +28.42 (c = 0.1, EtOAc); ee > 99.5% (detector UV 240 nm); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (d, J = 7.1 Hz, 3H), 3.76 (q, J = 7.0 Hz, 1H), 7.33−7.40 (m, 2H), 7.39−7.47 (m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 2.1 Hz, 1H), 12.35 (s, 1H); MS (ES, m/z) $C_{22}H_{15}Cl_2NO_3$ requires 411, found 366 $[M - H - CO_2]^-$; retention time on analytical chiral HPLC, 16.834 min.

*Data for (2R)-(6-chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)-propanoic acid [(−)-**15c**]:* $[\alpha]_D$ = −28.72 (c = 0.1, EtOAc), ee >99.5% (detector UV 240 nm); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (d, J = 7.1 Hz, 3H), 3.76 (q, J = 7.0 Hz, 1H), 7.33−7.40 (m, 2H), 7.39−7.47 (m, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.5 Hz, 2H), 8.22 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 2.1 Hz, 1H), 12.35 (s, 1H); MS (ES, m/z) $C_{22}H_{15}Cl_2NO_3$ requires 411, found 366 $[M - H - CO_2]^-$; retention time on analytical chiral HPLC, 23.442 min.

*Data for (2S)-(6-chloro-9-(oxazol-4-ylcarbonyl)-9H-carbazol-2-yl)propanoic acid [(+)-**15i**]:* $[\alpha]_D$ = +39.85 (c = 0.1, EtOAc); ee > 99.5% (detector UV 240 nm) ; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (d, J = 7.1 Hz, 3H), 3.82 (q, J = 7.0 Hz, 1H), 7.37 (dd, J = 8.1, 1.1 Hz, 1H), 7.44 (dd, J = 8.9, 2.2 Hz, 1H), 7.58−7.63 (m, 2H), 8.21 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 2.1 Hz, 1H), 8.69 (s, 1H), 9.06 (s, 1H), 12.38 (s, 1H); MS (ES, m/z) $C_{19}H_{13}ClN_2O_3S$ requires 384, found 323 $[M - H - CO_2]^-$; retention time on analytical chiral HPLC, 24.425 min.

*Data for (2R)-(6-chloro-9-(oxazol-4-ylcarbonyl)-9H-carbazol-2-yl)propanoic acid [(−)-**15i**]:* $[\alpha]_D$ = −39.38 (c = 0.1, EtOAc); ee >

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99.5% (detector UV 240 nm); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (d, J = 7.1 Hz, 3H), 3.82 (q, J = 7.0 Hz, 1H), 7.37 (dd, J = 8.1, 1.1 Hz, 1H), 7.44 (dd, J = 8.9, 2.2 Hz, 1H), 7.58−7.63 (m, 2H), 8.21 (d, J = 8.0 Hz, 1H), 8.33 (d, J = 2.1 Hz, 1H), 8.69 (s, 1H), 9.06 (s, 1H), 12.38 (s, 1H); MS (ES, m/z) $C_{19}H_{13}ClN_2O_3S$ requires 384, found 323 [M − H − CO_2]$^-$; retention time on analytical chiral HPLC, 37.257 min.

In Vitro Assays. FAAH activity was measured by incubation of [^{3}H]anandamide (1 μM cold AEA and 0.6 nM (1 mCi/mL) [^{3}H]AEA (arachidonyl[1-^{3}H]ethanolamine), specific activity 60 Ci/mmol; American Radiolabeled Chemicals, Inc., St. Louis, MO) for 30 min at 37 °C in the presence of 50 μg of protein per sample of total rat brain homogenates in assay buffer (50 mM Tris, pH 7.4, 0.05% fatty acid free BSA). The reaction was stopped with cold 1:1 $CHCl_3$/MeOH. The aqueous phase was counted by liquid scintillation (Microbeta2 Lumijet, Perkin-Elmer Inc., Waltham, MA; adapted from Kathuria et al., 2003). Inhibitors were preincubated with the enzyme preparation at the appropriate concentration for 10 min prior to substrate addition.

COX activity was measured using a commercial enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI). The manufacturer protocol was followed except for the substrate concentration. Briefly, inhibitors were preincubated with either ovine COX-1 or human COX-2 for 10 min at 37 °C, and the reaction was carried out in the presence of 5 μM arachidonic acid for 2 min at 37 °C. The reaction was stopped with hydrochloric acid, and COX-derived PGH_2 was then converted to PGF2α with $SnCl_2$. The PGF2α product was then quantified via enzyme immunoassay (EIA) using a PG-specific antibody and competing with a PG−acetylcholinesterase conjugate. Absorbance was measured at 412 nM with a Tecan Infinite M200 plate reader (Tecan Group Ltd., Männedorf, Switzerland), and data were processed according to the manufacturer's instructions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Percentage distribution of some molecular properties of the 382 COX inhibitors and putative binding mode to FAAH of some known COX inhibitors, including carprofen and some of its derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

FAAH, fatty acid amide hydrolase; PGE2, prostaglandin E2; DMAP, 4-(dimethylamino)pyridine; POX, peroxidase

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