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## Identification and characterization of carprofen as a multi-target FAAH/COX inhibitor

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### 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 non-steroid anti-inflammatory drug, carprofen, as a multi-target-directed ligand that simultaneously inhibits cyclooxygenase-1 (COX-1), COX-2 and fatty acid amide hydrolase (FAAH). Additionally, we synthesized and tested several racemic derivatives of carprofen, sharing this multi-target activity. This may result in improved analgesic efficacy and reduced side effects (Naidu, et al (2009) *J Pharmacol Exp Ther* 329, 48-56; Fowler, C.J. et al. (2012) *J Enzym Inhib Med Chem* Jan 6; Sasso, et al (2012) *Pharmacol Res* 65, 553). The new compounds are among the most potent multi-target FAAH/COXs 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.<sup>1-7</sup> 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.

Non-steroidal 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.<sup>8,9</sup> These enzymes convert arachidonic acid into prostaglandins and thromboxane, which are important physiological and

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#### Supporting Information

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

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.<sup>10</sup> There are several well-known classes of NSAIDs, which are either non-selective for COX-1 and COX-2 or selective for COX-2.<sup>11</sup> Both classes exert, however, a number of potentially serious side effects.<sup>12</sup> In the gastrointestinal tract, COX-1 inhibition blocks the synthesis of tissue-protecting prostaglandins such as PGE<sub>2</sub>, 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.<sup>13, 14</sup>

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.<sup>15-19</sup> 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.<sup>20-22</sup> 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.<sup>23, 24</sup> Several classes of FAAH inhibitors have been discovered during the last decade – including  $\alpha$ -ketoheterocycles, carbamate-, piperidine- and piperazine urea based molecules – some of which are undergoing pre-clinical and clinical studies.<sup>25-31</sup>

Several *in vivo* studies suggest that the simultaneous inhibition of COX and FAAH activities produces super-additive pharmacological effects and lowered toxicity in animal models. Naidu et al. showed that the FAAH inhibitor URB597<sup>32</sup> and the non-selective COX inhibitor diclofenac act synergistically to reduce visceral pain in mice.<sup>33</sup> Similar results were obtained by Sasso et al. using the peripherally restricted FAAH inhibitor URB597 and the NSAID indomethacin.<sup>34</sup> Importantly, both studies showed that FAAH blockade lowers the ulcerogenic activity of COX inhibitors.<sup>11</sup> These findings suggest that multi-target-directed-ligands<sup>35</sup> able to inhibit simultaneously 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.<sup>35-38</sup> 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 multi-target inhibitors that show improved potency compared to previously reported mixed FAAH/COXs compounds.<sup>23, 24, 39</sup> We used docking calculations to identify putative FAAH/COXs inhibitors starting from known COX-targeting drugs. *In vitro* pharmacological tests identified carprofen (Figure 1) as a multi-target FAAH/COXs hit. Based on this finding, we designed several carprofen derivatives that showed significant multi-target inhibitory activity, highlighting the potential of the carprofen scaffold as a source for new effective and safe analgesics.

## Results

### Identification of carprofen as a multi-target FAAH/COX inhibitor

We selected 382 COXs inhibitors retrieved from DrugBank<sup>40</sup> and DUD<sup>41</sup> 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). Based on their commercial availability, we purchased 25 compounds for testing (see Figure 2). These molecules were also clustered according to their topological distance and physico-chemical 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 multi-target FAAH/COX inhibitor among the 25 selected compounds. The *in vitro* results identified carprofen (**1**) as the best multi-target FAAH/COXs inhibitor in the set of compounds tested. Carprofen inhibited FAAH with a median effective concentration ( $IC_{50}$ ) = 79  $\mu$ M, COX-1 with an  $IC_{50}$  = 22  $\mu$ M and COX-2 with an  $IC_{50}$  = 4  $\mu$ M. As the profile against the three targets turned out to be rather balanced, compound **1** emerged as a promising starting point for multi-target FAAH/COXs lead discovery.

## Chemistry

Several possible chemical variations of **1** were considered (Scheme 1). The preparation of the corresponding *des*-chlorinated 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, respectively with ethanolamine and aniline.

The functionalization of the nitrogen atom of **1** was achieved using ester **3** as 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 *para*-chlorophenyl moiety, the methyl ester hydrolysis was performed in acidic media (due to the observed cleavage of the urea bond of **9d** under basic conditions) to provide **10d** in a good 80 % yield.

The carbamate derivative **12** was prepared under standard conditions (Scheme 3). Compound **3** was reacted in THF with hexyl chloroformate, in presence of  $Et_3N$  and DMAP, to give intermediate **11**, which was further hydrolyzed under acidic condition 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.<sup>42</sup> We therefore decided to protect **1** as an *O*-benzyl ester, using standard reaction conditions (BnBr, K<sub>2</sub>CO<sub>3</sub>, DMF).<sup>43</sup> The acylation reactions of the benzyl ester **13** proceeded well in MeCN in the presence of DMAP and Et<sub>3</sub>N, with various acyl chlorides, to afford esters **14**.

The last debenzoylation step to obtain the corresponding acids **15** was performed using the H-Cube apparatus, under optimized conditions in order to reduce the formation of the undesired *des*-chloro 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 H<sub>2</sub> pressure (H-Cube “full H<sub>2</sub> mode”). In this case, complete conversion of the benzyl ester **14** was observed and the expected **15** was formed in 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 H<sub>2</sub> pressure, in a closed-loop system with the H-Cube (where the out-coming solution was directly re-injected in the in-coming 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 **15**; the reaction was stopped when compounds **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 starting material and to carry the hydrolysis in acidic media, 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<sub>50</sub> 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<sub>50</sub> = 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 COXs 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<sub>50</sub> = 3 μM) compared to **1**. Based on 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<sub>50</sub> = 15 μM against FAAH, while a benzyl group on the carbazole nitrogen gave a 3-fold improvement in FAAH inhibition compared to **1** (**6b**, IC<sub>50</sub> = 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<sub>50</sub> = 33 μM).

Conversely, the *p*-Cl derivative **6d** and the *p*-OCH<sub>3</sub> derivative **6e** showed greater potency against FAAH, with IC<sub>50</sub> values of 10 μM and 8 μM, respectively. Moreover, we observed that the *para*-substitution was preferential: the *p*-OCH<sub>3</sub> derivative **6e** was more potent against FAAH (IC<sub>50</sub> = 8 μM) than the *m*-OCH<sub>3</sub> derivative **6f** (IC<sub>50</sub> = 32 μM) and the *o*-OCH<sub>3</sub> derivative **6g** (which showed no activity at 30 μM). Within the series of sulfonamides, **8a** and **8b** were active against FAAH, displaying IC<sub>50</sub> values of 50 μM 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.<sup>43</sup>

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<sub>50</sub> = 3 μM), bearing a linear C<sub>6</sub> chain. Shorter linear chains, from C<sub>1</sub> to C<sub>4</sub>, showed no inhibition on FAAH, as exemplified by **10b** and **10c**. Longer chains brought to less potent FAAH inhibitors, but interestingly led to an increase in COX-1 inhibition. For example **10d** showed an IC<sub>50</sub> = 27 μM against FAAH and an IC<sub>50</sub> = 15 μM against COX-1. Compound **10e** (bearing a 4-chlorobenzyl moiety), showed weak potency against FAAH (IC<sub>50</sub> = 102 μM), and an IC<sub>50</sub> value of 41 μM against COX-1. *N,N*-disubstituted derivatives or ureas bearing 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<sub>50</sub> = 32 μM) compared to its carbamate analog **10a** (IC<sub>50</sub> = 3 μM), while it showed an IC<sub>50</sub> = 17 μM on COX-1.

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

In light of these promising results, we came to the conclusion that a carbonyl function linked to the nitrogen atom of the carbazole ring of **1** could be important to obtain a multi-target 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*-F-benzoyl and *p*-OCH<sub>3</sub>-benzoyl analogs **15d** and **15e**, which showed IC<sub>50</sub> values on FAAH of 31 μM 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*-alkyls series, the *para* substitution was the most tolerated. The *m*-Cl derivative **15f** (FAAH IC<sub>50</sub> = 20 μM), and the *o*-Cl derivative **15g** (FAAH IC<sub>50</sub> = 60 μM) retained a similar inhibitory potency for FAAH compared to **15c**, while inhibitory activity against COXs was lost. A total loss of activity for the three enzymes was observed when we tried to combine the most tolerated *para*- 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** showed to be well tolerated, with FAAH IC<sub>50</sub> = 85 μM, COX-1 IC<sub>50</sub> = 30 μM and COX-2 IC<sub>50</sub> = 28 μM. Compound **15j**, bearing a 4-imidazole ring, showed FAAH IC<sub>50</sub> = 6 μM and COX-1 IC<sub>50</sub> = 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 COXs 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 active against the three targets. For **15c** and **15i**, the *S*(+) enantiomer was the only 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.<sup>44, 45</sup> In addition, the inactivity of *R*(-) enantiomers of **1**, **15c** and **15i** against COXs was expected, in agreement with previous results showing a preferential COXs inhibition by *S*(+) enantiomers.<sup>46</sup>

## Discussion

The present results identify carprofen (compound **1**) as a multi-target 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  $\mu\text{M}$  range (see Tables 1 and 2). The ability of NSAIDs to inhibit anandamide hydrolysis has been previously described by Fowler and coworkers in several informative studies.<sup>39, 47, 48</sup> 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 multi-target FAAH/COX inhibitor.<sup>23, 24, 39, 49</sup> This compound displayed an  $\text{IC}_{50}$  value for FAAH of  $\sim 0.5 \mu\text{M}$  (in EtOH), and  $\text{IC}_{50}$  values for COX-1 of  $\sim 60 \mu\text{M}$  and  $\sim 240 \mu\text{M}$  in DMSO and ethanol, respectively. These data for COXs inhibition were generated using an oxygen electrode assay for peroxidase (POX) activity.<sup>24</sup> Under these experimental conditions, ibu-am5 was inactive against COX-2. However, if anandamide was used as COX-2 substrate in the assay, instead of arachidonic acid, ibu-am5 showed a COX-2  $\text{IC}_{50} \sim 20 \mu\text{M}$ , suggesting a substrate-dependency in COX-2 inhibition.<sup>24</sup> Under our present experimental conditions, ibu-am5 inhibited FAAH with an  $\text{IC}_{50}$  of  $66 \mu\text{M}$ . COX inhibition was measured with an enzyme immunoassay using a prostaglandin-specific antibody (see Methods), which yielded a  $\text{IC}_{50}$  for COX-1 of  $170 \mu\text{M}$ . The potency toward COX-2 was very low, as ibu-am5 produced only 30% inhibition at  $30 \mu\text{M}$ . We also tested ibuprofen as an additional reference compound, which showed  $\text{IC}_{50}$  of  $5 \mu\text{M}$  and  $36 \mu\text{M}$  against COX-1 and COX-2, respectively, while it was very weak against FAAH, in reasonably good agreement with previous findings.<sup>47, 50</sup>

Therefore, carprofen and its racemic derivatives described in the present study are among the most potent multi-target FAAH/COX inhibitors reported so far. These molecules also show a well-balanced multi-target profile, implying that they may provide a starting point for the discovery of new FAAH/COXs inhibitors to treat pain, inflammation and potentially, as recently discussed, cancer.<sup>51, 52</sup> 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.<sup>34</sup>

Despite being evolutionary unrelated, FAAH and COXs share several structural similarities, which might help in the rationalization of the multi-target 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.<sup>49</sup> Because of the presence of such common structural features, useful to discuss also the basis of biological promiscuity,<sup>53</sup> 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 on 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.<sup>9</sup> 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). Notably, **1** is a small molecule bearing fragment-like physicochemical features.<sup>54</sup>

Finally, it is worth mentioning that the multi-target FAAH/COXs 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.<sup>55</sup> 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.<sup>34</sup>

## Conclusions

We reported on the identification of multi-target inhibitors that block simultaneously 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 multi-target FAAH/COXs 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 inhibitors dataset

A large collection of known COXs inhibitors was retrieved, in a ready-to-dock format, from DUD<sup>41</sup> and from DrugBank.<sup>40</sup> The grand total of unique molecules collected, after eliminating double occurrences, was 382. Some of these are currently marketed drugs mostly in use as anti-inflammatory agents, while others have been reported to inhibit at least one of the two COX isoforms, with diverse potencies. For years, COXs 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, non-selective 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 non covalent 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'.<sup>56</sup>

The dataset 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), oxicames (e.g. tenoxicam) as well as 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 COXs 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), such as in this case. To better characterize the molecular diversity in the dataset, we looked at specific physico-chemical features of each compound. The distribution observed for nine common physico-chemical descriptors further describes the diversity found over the entire dataset. 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 HB donors of either 0 or 2 (see Figure S1).

### 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,  $T_c$ , where  $T_c = c/(a + b - c)$ , where  $c$  counts the common bits *on* in molecule 1 and molecule 2,  $a$  counts the bits *on* in molecule 1, and  $b$  counts the bits *on* in 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 databank (PDBid 1mt5)<sup>57</sup> and chain A of the X-ray structure of COX-2, in complex with flurbiprofen (PDBid 3pgh)<sup>58</sup> were used for the docking study. Missing sidechains were added through SCWRL 4.0.<sup>59</sup> Then, water and co-crystallized ligands were removed, and the catalytic serine (S241) was treated as negatively charged. The so-obtained structures were energy minimized with NAMD 2.6<sup>60</sup> program until a 0.3 Å RMSD convergence criterion on heavy atoms was reached. The Amber force field parm99SB<sup>61</sup> was employed throughout. The final protein models and the selected known COXs inhibitors were then converted through the Python scripts available in the ADT suite of programs<sup>62</sup> into a suitable format for AutoDock Vina v. 1.0.<sup>63</sup>

AutoDock Vina v.1.0<sup>63</sup> was used to dock 382 known COXs inhibitors at the FAAH binding site. The search volume, centered on the phosphate atom of the co-crystallized 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 (approx. 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 we eventually purchased 25 compounds for experimental testing (see Figure 2). Some molecules such as acetaminophen and aspirin that exceeded the rank threshold (rank no. 350 and 326, respectively) were considered because they were readily available. Despite their low scores, we also included molecules belonging to an underrepresented 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 were indicated as follows: acetyl chloride (AcCl), acetonitrile (MeCN), ammonium chloride (NH<sub>4</sub>Cl), benzyl bromide (BnBr), carbonyldiimidazole (CDI), cesium carbonate (Cs<sub>2</sub>CO<sub>3</sub>), cyclohexane (Cy), chloroform (CHCl<sub>3</sub>), dichloromethane (DCM), dimethylsulfoxide (DMSO), diethyl ether (Et<sub>2</sub>O), *N,N*-diisopropylethylamine (DIPEA), 4-(dimethylamino)-pyridine (DMAP), di-*tert*-butyl dicarbonate (Boc<sub>2</sub>O), ethanol (EtOH), ethyl acetate (EtOAc), hydrochloric acid (HCl), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), iodomethane (MeI), *N,N*-dimethylformamide (DMF), lithium hydroxide (LiOH), magnesium sulfate (MgSO<sub>4</sub>), methanol (MeOH), sodium bicarbonate (NaHCO<sub>3</sub>), tetrabutylammonium iodide (TBAI), tetrahydrofuran (THF), triethylamine (Et<sub>3</sub>N), trifluoroacetic acid (TFA).

Automated column chromatography purifications were done using a Teledyne ISCO apparatus (CombiFlash<sup>®</sup> Rf) with pre-packed silica gel columns of different sizes (from 4 g until 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 pre-coated 0.05 mm TLC plates (SIL G-50 UV<sub>254</sub>). Hydrogenation reactions were performed using H-Cube<sup>®</sup> continuous hydrogenation equipment (SS-reaction line version), employing disposable catalyst cartridges (CatCart<sup>®</sup>) preloaded with the required heterogeneous catalyst. Microwave heating was performed using Explorer<sup>®</sup>-48 positions instrument (CEM). NMR experiments were run on a Bruker Avance III 400 system (400.13 MHz for <sup>1</sup>H, and 100.62 MHz for <sup>13</sup>C), equipped with a BBI probe and Z-gradients. Spectra were acquired at 300 K, using deuterated dimethylsulfoxide (DMSO-*d*<sub>6</sub>) or deuterated chloroform (CDCl<sub>3</sub>) as solvents. Chemical shifts for <sup>1</sup>H and <sup>13</sup>C spectra were recorded in parts per million using the residual non-deuterated solvent as the internal standard (for DMSO-*d*<sub>6</sub>: 2.50 ppm, <sup>1</sup>H; 39.52 ppm, <sup>13</sup>C; for CDCl<sub>3</sub>: 7.26 ppm, <sup>1</sup>H and 77.16 ppm, <sup>13</sup>C). Data are reported as follows: chemical shift (ppm), multiplicity (indicated as: bs, broad signal; s, singlet; d, doublet; t, triplet; q, quartet; p, quintet; sx, sextet; m, multiplet and combinations thereof), coupling constants (*J*) in Hertz (Hz) and integrated intensity. UPLC/MS analyses were run on a Waters ACQUITY UPLC/MS system consisting of a SQD (Single Quadrupole Detector) Mass Spectrometer equipped with an Electrospray Ionization interface and a Photodiode Array Detector. PDA range was 210-400 nm. Analyses were performed on an ACQUITY UPLC BEH C18 column (50×2.1 mmID, particle size 1.7 μm) with a VanGuard BEH C18 pre-column (5×2.1 mmID, particle size 1.7 μm). Mobile phase was either 10 mM NH<sub>4</sub>OAc in H<sub>2</sub>O at pH 5 adjusted with AcOH (A) and 10 mM NH<sub>4</sub>OAc in MeCN-H<sub>2</sub>O (95:5) at pH 5 (B) or 5 mM NH<sub>4</sub>OAc + 0.25 % AcOH in H<sub>2</sub>O (A) and 5 mM NH<sub>4</sub>OAc + 0.25 % AcOH in MeOH (B) for compounds **5**. Electrospray ionization in positive and negative mode was applied. Purifications by preparative HPLC/MS were run on a Waters Autopurification system consisting of a 3100 Single Quadrupole Mass Spectrometer equipped with an Electrospray Ionization interface and a 2998 Photodiode Array Detector. HPLC system included a 2747 Sample Manager, 2545 Binary Gradient

Module, System Fluidic Organizer and 515 HPLC Pump. PDA range was 210–400 nm. Purifications were performed on a XBridge™ Prep C18 OBD column (100×19 mmID, particle size 5 μm) with a XBridge™ Prep C18 (10×19 mmID, particle size 5 μm) Guard Cartridge. Mobile phase was 10 mM NH<sub>4</sub>OAc in H<sub>2</sub>O at pH 5 adjusted with AcOH (A) and 10 mM NH<sub>4</sub>OAc in MeCN–H<sub>2</sub>O (95:5) at pH 5 (B). Electrospray ionization in positive and negative mode 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. PDA range was 210–400 nm. Analyses were performed isocratic on a Daicel ChiralPak AD column (250×4.6 mmID, particle size 10 μm). 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, 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 (250×10mmID, particle size 10 μm). 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 g/100 mL using EtOAc or MeOH as a solvent and a 1 dm cell. All final compounds displayed 95% purity as determined by NMR and UPLC/MS analysis.

**2-(9H-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 1 mL/min flow rate, room temperature and H<sub>2</sub> atmosphere (1 atm) (full H<sub>2</sub> mode). After three consecutive runs, the solvent was removed *in vacuo* and the crude oil was purified by preparative HPLC to yield the corresponding *des*-chlorinated compound **2** (37 mg, 40 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>15</sub>H<sub>13</sub>NO<sub>2</sub> requires 239, found 240 [M+H]<sup>+</sup>.

**Methyl 2-(6-chloro-9H-carbazol-2-yl)propanoate 3**—To a suspension of carprofen **1** (1.055 g, 3.85 mmol) in MeOH (40 mL) was added H<sub>2</sub>SO<sub>4</sub> (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<sub>3</sub> solution (3 × 20 mL) and brine (10 mL). After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo* to give **3** (1.15 g, quant.) as a pale yellow solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>16</sub>H<sub>14</sub>ClNO<sub>2</sub> requires 287, found 286 [M-H]<sup>-</sup>.

**2-(6-chloro-9H-carbazol-2-yl)-N-(2-hydroxyethyl)propanamide 4a**—To a solution of carprofen **1** (112 mg, 0.41 mmol, 1 eq) in pyridine (2 mL) was added CDI (133 mg, 0.82 mmol, 2 eq) portionwise. The mixture was stirred for 2 h at room temperature, before addition of ethanolamine (50 μL, 0.82 mmol, 2 eq) and then the reaction was heated at 55°C overnight. Pyridine was then evaporated, the residue taken up in EtOAc and washed with a saturated aqueous NH<sub>4</sub>Cl solution (5mL). After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by column chromatography (DCM/MeOH) to give amide **4a** (110 mg, 85 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>17</sub>H<sub>17</sub>ClN<sub>2</sub>O<sub>2</sub> requires 316, found 317 [M+H]<sup>+</sup>.

**2-(6-chloro-9H-carbazol-2-yl)-N-phenylpropanamide 4b**—To a solution of carprofen **1** (126 mg, 0.46 mmol, 1 eq) in pyridine (2 mL) was added CDI (149 mg, 0.92 mmol, 2 eq) portionwise. The mixture was stirred for 2 h at room temperature, before addition of aniline (84  $\mu$ L, 0.92 mmol, 2 eq) and then the reaction was heated at 55 °C overnight. Pyridine was then evaporated, the residue taken up in EtOAc and washed with a saturated aqueous NH<sub>4</sub>Cl solution (5 mL). After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by column chromatography (DCM/MeOH) to give amide **4b** (138 mg, 86 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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) C<sub>21</sub>H<sub>17</sub>ClN<sub>2</sub>O requires 348, found 349 [M+H]<sup>+</sup>.

### General procedure A

To a solution of **3** in MeCN (0.05 M solution) were successively added the alkyl halide (3 to 5 eq) and Cs<sub>2</sub>CO<sub>3</sub> (5 eq). The mixture was heated under reflux overnight. After cooling down to room temperature, the mixture was filtered. EtOAc and H<sub>2</sub>O were added to the filtrate. After separation, the organic phase was dried over MgSO<sub>4</sub> 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<sub>2</sub>CO<sub>3</sub> (756 mg, 1.96 mol) afforded the methylated product **5a** (118 mg, quant.) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  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) C<sub>17</sub>H<sub>16</sub>ClNO<sub>2</sub> requires 301, found 302 [M+H]<sup>+</sup>.

**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<sub>2</sub>CO<sub>3</sub> (692 mg, 1.79 mmol) afforded the benzylated product **5b** (55 mg, 40 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  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) C<sub>23</sub>H<sub>20</sub>ClNO<sub>2</sub> requires 377, found 378 [M+H]<sup>+</sup>.

**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<sub>2</sub>CO<sub>3</sub> (785 mg, 1.94 mmol) afforded the 4-cyanobenzylated product **5c** (149 mg, 95 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  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) C<sub>24</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>2</sub> requires 402, found 401 [M-H]<sup>-</sup>.

**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<sub>2</sub>CO<sub>3</sub> (816 mg, 2.11 mmol) afforded the 4-methoxybenzylated product **5e** (99 mg, 55 %) as a yellow oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  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)  $C_{24}H_{22}ClNO_3$  requires 407, found 408  $[M+H]^+$ .

### General procedure B

To a solution of the methyl ester in a 1:1:1 MeOH:THF:H<sub>2</sub>O (0.03 M solution) was added LiOH (4 eq). The mixture was stirred at room temperature overnight, then the organic solvents were evaporated. The aqueous solution was then acidified to pH 1 with 6M HCl. The precipitate formed was either 1) filtered, washed with H<sub>2</sub>O and dried under vacuum or 2) redissolved in EtOAc, dried over MgSO<sub>4</sub> 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 mg, 85 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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)  $C_{16}H_{14}ClNO_2$  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. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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)  $C_{22}H_{18}ClNO_2$  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. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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)  $C_{23}H_{17}ClN_2O_2$  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 eq) in MeCN (5 mL) were successively added 4-chlorobenzyl bromide (308 mg, 1.50 mmol, 3 eq), Cs<sub>2</sub>CO<sub>3</sub> (814 mg, 5 mmol, 5 eq) and TBAI (92 mg, 0.25 mmol, 0.5 eq). 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<sub>2</sub>O solution (3 mL). LiOH (24 mg, 1 mmol, 2 eq) was added and the mixture was stirred at room temperature overnight. The solution was then acidified with 2M HCl and extracted with EtOAc. After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was crystallised in Et<sub>2</sub>O to obtain acid **6d** (64 mg, 32 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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)  $C_{22}H_{17}Cl_2NO_2$  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. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>23</sub>H<sub>20</sub>ClNO<sub>3</sub> requires 393, found 348 [M-H-CO<sub>2</sub>]<sup>-</sup>.

**2-(6-chloro-9-(3-methoxybenzyl)-9H-carbazol-2-yl)propanoic acid 6f**—To a solution of **3** (144 mg, 0.5 mmol, 1 eq) in MeCN (5 mL) were successively added 3-methoxybenzyl bromide (0.21, 1.50 mmol, 3 eq), Cs<sub>2</sub>CO<sub>3</sub> (814 mg, 5 mmol, 5 eq) and TBAI (92 mg, 0.25 mmol, 0.5 eq). 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<sub>2</sub>O solution (3 mL). LiOH (24 mg, 1 mmol, 2 eq) was added and the mixture was stirred at room temperature overnight. The solution was then acidified with 2M HCl and extracted with EtOAc. After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by preparative HPLC to obtain acid **6f** (42 mg, 21 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>23</sub>H<sub>20</sub>ClNO<sub>3</sub> requires 393, found 348 [M-H-CO<sub>2</sub>]<sup>-</sup>.

**2-(6-chloro-9-(2-methoxybenzyl)-9H-carbazol-2-yl)propanoic acid 6g**—To a solution of **3** (144 mg, 0.5 mmol, 1 eq) in MeCN (5 mL) were successively added 2-methoxybenzyl bromide (235 mg, 1.50 mmol, 3 eq), Cs<sub>2</sub>CO<sub>3</sub> (814 mg, 5 mmol, 5 eq) and TBAI (92 mg, 0.25 mmol, 0.5 eq). 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<sub>2</sub>O solution (3 mL). LiOH (24 mg, 1 mmol, 2 eq) was added and the mixture was stirred at room temperature overnight. The solution was then acidified with 2M HCl and extracted with EtOAc. After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by preparative HPLC to obtain acid **6g** (46 mg, 23 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>23</sub>H<sub>20</sub>ClNO<sub>3</sub> requires 393, found 348 [M-H-CO<sub>2</sub>]<sup>-</sup>.

**Methyl 2-(6-chloro-9-(hexylsulfonyl)-9H-carbazol-2-yl)propanoate 7a**—To a solution of **3** (105 mg, 0.36 mmol, 1 eq) in THF (5 mL) were successively added 1-hexanesulfonyl chloride (0.17 mL, 1.09 mmol, 3 eq), DMAP (133 mg, 1.09 mmol, 3 eq) and Et<sub>3</sub>N (0.15 mmol, 1.09 mmol, 3 eq). 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. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>22</sub>H<sub>26</sub>ClNO<sub>4</sub>S requires 435, found 436 [M+H<sup>+</sup>], 453 [M+NH<sub>4</sub><sup>+</sup>].



**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 eq) in THF (5 mL) were successively added 4-chlorobenzene sulfonyl chloride (241 mg, 1.14 mmol, 3 eq), DMAP (140 mg, 1.14 mmol, 3 eq) and Et<sub>3</sub>N (0.16 mL, 1.14 mmol, 3 eq). 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. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>22</sub>H<sub>17</sub>Cl<sub>2</sub>NO<sub>4</sub>S requires 461, found 460, 462 [M-H]<sup>-</sup>.

**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<sub>2</sub>O, furnished acid **8a** (15 mg, 28 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>21</sub>H<sub>24</sub>ClNO<sub>4</sub>S requires 421, found 376 [M-H-CO<sub>2</sub>]<sup>-</sup>, 420 [M-H]<sup>-</sup>.

**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<sub>2</sub>O, furnished acid **8b** (51 mg, 40 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 1.49 (d, *J* = 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) C<sub>21</sub>H<sub>15</sub>Cl<sub>2</sub>NO<sub>4</sub>S requires 447, found 402, 404 [M-H-CO<sub>2</sub>]<sup>-</sup>, 446, 448 [M-H]<sup>-</sup>.

**Methyl 2-(6-chloro-9-(hexylcarbamoyl)-9H-carbazol-2-yl)propanoate 9a**

—To a solution of **3** (100 mg, 0.35 mmol, 1 eq) in THF (5 mL) were successively added hexyl isocyanate (0.10 mL, 0.70 mmol, 2 eq), DMAP (85 mg, 0.70 mmol, 2 eq) and Et<sub>3</sub>N (0.15 mL, 1.05 mmol, 3 eq). The mixture was heated under reflux for 60 h and then concentrated. The residue was taken up in EtOAc and a saturated aqueous NH<sub>4</sub>Cl solution (5 mL) was added. After separation, the organic phase was dried over MgSO<sub>4</sub> 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. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>23</sub>H<sub>27</sub>ClN<sub>2</sub>O<sub>3</sub> requires 414, found 415 [M+H]<sup>+</sup>, 433 [M+NH<sub>4</sub>]<sup>+</sup>.

**Methyl 2-[6-chloro-9-(methylcarbamoyl)carbazol-2-yl]propanoate 9b**

—To a solution of Boc<sub>2</sub>O (0.392 mg, 1.79 mmol, 3 eq) in MeCN (3 mL) were successively added DMAP (219 mg, 1.79 mmol, 3 eq) and methylamine (2M solution in THF, 0.89 mL, 1.79 mmol, 3 eq). The mixture was stirred for 30 min at room temperature and then added to a solution of **3** (170 mg, 0.60 mmol, 1 eq) 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<sub>4</sub>Cl solution (5 mL) were then added. After separation, the organic phase was dried over MgSO<sub>4</sub> 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. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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)  $C_{18}H_{17}ClN_2O_3$  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 eq) in THF (5 mL) were successively added butyl isocyanate (0.12 mL, 1.09 mmol, 3 eq), DMAP (133 mg, 1.09 mmol, 3 eq) and  $Et_3N$  (0.15 mL, 1.09 mmol, 3 eq). 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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{21}H_{23}ClN_2O_3$  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 eq) in THF (5 mL) were successively added octyl isocyanate (0.19 mL, 1.10 mmol, 3 eq), DMAP (135 mg, 1.10 mmol, 3 eq) and  $Et_3N$  (0.15 mL, 1.10 mmol, 3 eq). 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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{25}H_{31}ClN_2O_3$  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 eq) in THF (5 mL) were successively added 4-chlorophenyl isocyanate (0.16 mL, 1.21 mmol, 3 eq), DMAP (148 mg, 1.21 mmol, 3 eq) and  $Et_3N$  (0.17 mL, 1.21 mmol, 3 eq). 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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{23}H_{18}Cl_2N_2O_3$  requires 440, found 441, 443  $[M+H]^+$ , 458, 460  $[M+NH_4]^+$ .

**Methyl 2-[6-chloro-9-[hexyl(methyl)carbamoyl]carbazol-2-yl]propanoate 9f**—To a solution of **3** (124 mg, 0.43 mmol, 1 eq) in THF (5 mL) were successively added *N*-hexyl-*N*-methyl-carbamoyl chloride (460 mg, 2.58 mmol, 6 eq, freshly prepared from *N*-methylhexylamine (1.0 eq) and triphosgene (0.33 eq) in presence of pyridine (1.0 eq) in dry DCM), DMAP (158 mg, 1.29 mmol, 3 eq) and  $Et_3N$  (0.18 mL, 1.27 mmol, 3 eq). 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 colourless oil.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  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)  $C_{24}H_{29}ClN_2O_3$  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 eq) in THF (5 mL) were successively added cyclohexyl isocyanate (0.10 mL, 0.70 mmol, 2 eq), DMAP (85 mg, 0.70 mmol, 2 eq) and  $Et_3N$  (0.15 mL, 1.05 mmol, 3 eq). 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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{23}H_{25}ClN_2O_3$  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.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  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)  $C_{22}H_{25}ClN_2O_3$  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 6M 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.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  1.45 (d,  $J = 7.1$  Hz, 3H), 2.94 (d,  $J = 4.4$  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)  $C_{17}H_{15}ClN_2O_3$  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.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  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)  $C_{20}H_{21}ClN_2O_3$  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.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  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)  $C_{24}H_{29}ClN_2O_3$  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 6M HCl (5 mL). The solution was stirred for 5 days at room temperature. EtOAc (10 mL) and H<sub>2</sub>O (5 mL) were then added. After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was triturated with Et<sub>2</sub>O/pentane to furnish acid **10e** (105 mg, 80 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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)  $C_{22}H_{16}Cl_2N_2O_3$  requires 426, found 427, 429  $[M+H]^+$ , 444, 446  $[M+NH_4]^+$ .

**2-[6-chloro-9-[hexyl(methyl)carbamoyl]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 colourless oil. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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)  $C_{23}H_{27}ClN_2O_3$  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<sub>2</sub>O, furnished acid **10g** (51 mg, 89 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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)  $C_{22}H_{23}ClN_2O_3$  requires 398, found 399  $[M+H]^+$ .

**Hexyl 6-chloro-2-(1-methoxy-1-oxopropan-2-yl)-9H-carbazole-9-carboxylate 11**

—To a solution of **3** (140 mg, 0.49 mmol, 1 eq) in THF (5 mL) were successively added hexyl chloroformate (freshly prepared, 215 mg, 1.47 mmol, 3 eq), DMAP (179 mg, 1.47 mmol, 3 eq) and Et<sub>3</sub>N (0.20 mL, 1.47 mmol, 3 eq). 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. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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)  $C_{23}H_{26}ClNO_4$  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 6M HCl (2.5 mL). The solution was stirred for 5 days at room temperature. EtOAc (10 mL) and H<sub>2</sub>O (5 mL) were then added. After separation, the organic phase was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was purified by column chromatography (Cy/EtOAc) to furnish acid **111** (46 mg, 55 %) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.89 (t,  $J = 6.9$  Hz, 3H), 1.31-1.39 (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)  $C_{22}H_{24}ClNO_4$  requires 401, found 400  $[M-H]^-$ , 356  $[M-H-CO_2]^-$ .

**Benzyl 2-(6-chloro-9H-carbazol-2-yl)propanoate 13.**<sup>43</sup>—To a solution of **1** (773 mg, 2.82 mmol, 1 eq) in DMF (10 mL) was added  $K_2CO_3$  (1.17 g, 8.47 mmol, 3 eq). The mixture was stirred at room temperature for 30 min, then BnBr (0.37 mL, 3.10 mmol, 1.1 eq) 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%).  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{22}H_{18}ClNO_2$  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 eq, either obtained from commercial source or freshly prepared from the corresponding acid), DMAP (3 eq) and  $Et_3N$  (3 eq). 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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{24}H_{20}ClNO_3$  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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{29}H_{22}ClNO_3$  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.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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), 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)  $C_{29}H_{21}Cl_2NO_3$  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<sub>3</sub>N (0.11 mL, 0.80 mmol) furnished the 4-fluorobenzoyl derivative **14d** (104 mg, 88 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>29</sub>H<sub>21</sub>ClFNO<sub>3</sub> requires 485, found 486 [M+H<sup>+</sup>].

**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<sub>3</sub>N (0.16 mL, 1.12 mmol) furnished the 4-methoxybenzoyl derivative **14e** (156 mg, 85 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>30</sub>H<sub>24</sub>ClNO<sub>4</sub> requires 497, found 498 [M+H<sup>+</sup>].

**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<sub>3</sub>N (0.13 mL, 0.96 mmol) furnished the 3-chlorobenzoyl derivative **14f** (124 mg, 77 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>29</sub>H<sub>21</sub>Cl<sub>2</sub>NO<sub>3</sub> requires 501, found 502 [M+H<sup>+</sup>], 519 [M+NH<sub>4</sub><sup>+</sup>].

**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<sub>3</sub>N (0.12 mL, 0.86 mmol) furnished the 2-chlorobenzoyl derivative **14g** (129 mg, 88 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>29</sub>H<sub>21</sub>Cl<sub>2</sub>NO<sub>3</sub> requires 501, found 502 [M+H<sup>+</sup>], 519 [M+NH<sub>4</sub><sup>+</sup>].

**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<sub>3</sub>N (0.14 mL, 1 mmol) furnished the 3,4-dichlorobenzoyl derivative **14h** (157 mg, 89 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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) C<sub>29</sub>H<sub>20</sub>Cl<sub>3</sub>NO<sub>3</sub> requires 535, found 536, 538 [M+H<sup>+</sup>], 553, 555 [M+NH<sub>4</sub><sup>+</sup>].

**Benzyl 2-(6-chloro-9-(oxazole-4-carbonyl)-9H-carbazol-2-yl)propanoate 14i—**

Following general procedure C, acylation of **13** (144 mg, 0.39 mmol) with 4-oxazole carbonyl chloride (freshly prepared from 4-oxazole carboxylic acid, 1.20 mmol), DMAP (145 mg, 1.20 mmol) and Et<sub>3</sub>N (0.16 mL, 1.20 mmol) furnished the 4-oxazole carbonyl derivative **14i** (135 mg, 76 %) as a white solid. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 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)  $C_{26}H_{19}ClN_2O_4$  requires 458, found 459  $[M+H]^+$ , 476  $[M+NH_4]^+$ .

**Tert-butyl 4-(2-(1-(benzyloxy)-1-oxopropan-2-yl)-6-chloro-9H-carbazole-9-carbonyl)-1H-imidazole-1-carboxylate 14j**—To a suspension of imidazole-4-carboxylic acid (143 mg, 1.28 mmol, 1 eq) in DMF (1.5 mL) were successively added  $Et_3N$  (0.35 mL, 2.56 mmol, 2 eq) and a solution of  $Boc_2O$  (307 mg, 1.08 mmol, 1.1 eq) 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 eq) followed by DMF (0.05 mL) were added. The solution was stirred for 2 h at room temperature and then concentrated *in vacuo* to provide the crude 1-Boc imidazole-4-carbonyl chloride as yellow oil. To a solution of **13** (120 mg, 0.33 mmol, 1 eq) in MeCN (3 mL) were successively added the crude acyl chloride described previously (1.28 mmol, 3.9 eq) in MeCN (2 mL), DMAP (156 mg, 1.28 mmol, 3.9 eq) and  $Et_3N$  (0.18 mL, 1.28 mmol, 3.9 eq). The mixture was stirred at room temperature for 2 h, 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*-Boc carbonylimidazole compound **14j** (46 mg, 25%) as a white solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{31}H_{28}ClN_3O_5$  requires 557, found 558  $[M+H]^+$ .

**Methyl 2-(6-chloro-9-(thiazole-4-carbonyl)-9H-carbazol-2-yl)propanoate 14k**—Following general procedure C, acylation of **3** (117 mg, 0.41 mmol) with thiazole-4-carbonyl 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 4-thiazole carbonyl derivative **14k** (115 mg, 71 %) as a white solid.  $^1H$  NMR (400 MHz,  $CDCl_3$ )  $\delta$  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)  $C_{20}H_{15}ClN_2O_3S$  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<sup>TM</sup> using a 1% Pd/C catalyst, at 1 mL/min flow rate, room temperature and  $H_2$  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.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ )  $\delta$  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)  $C_{17}H_{14}ClNO_3$  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<sup>TM</sup> using a 1 % or 5 % Pd/C catalyst, at 1 mL/min flow rate, room temperature and  $H_2$  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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{16}\text{ClNO}_3$  requires 377, found 378  $[\text{M}+\text{H}^+]$ , 395  $[\text{M}+\text{NH}_4^+]$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{15}\text{ClNO}_3$  requires 411, found 366  $[\text{M}-\text{H}-\text{CO}_2]^-$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{15}\text{ClFNO}_3$  requires 395, found 396  $[\text{M}+\text{H}^+]$ , 413  $[\text{M}+\text{NH}_4^+]$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{23}\text{H}_{18}\text{ClNO}_4$  requires 407, found 408  $[\text{M}+\text{H}^+]$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{15}\text{Cl}_2\text{NO}_3$  requires 411, found 412  $[\text{M}+\text{H}^+]$ , 434  $[\text{M}+\text{Na}^+]$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{15}\text{Cl}_2\text{NO}_3$  requires 411, found 412  $[\text{M}+\text{H}^+]$ , 429  $[\text{M}+\text{NH}_4^+]$ .

**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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{22}\text{H}_{14}\text{Cl}_3\text{NO}_3$  requires 445, found 400, 402  $[\text{M}-\text{H}-\text{CO}_2]^-$ .

**2-(6-chloro-9-(oxazole-4-carbonyl)-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.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{19}\text{H}_{13}\text{ClN}_2\text{O}_4$  requires 368, found 323  $[\text{M-H-CO}_2]^-$ .

**2-(6-chloro-9-(1H-imidazole-4-carbonyl)-9H-carbazol-2-yl)propanoic acid hydrochloride 15j**—To a solution of **14j** (84 mg, 0.15 mmol) in acetone (2 mL) 2M HCl was added (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<sup>TM</sup> using a 5 % Pd/C catalyst, at 1 mL/min flow rate, room temperature and  $\text{H}_2$  atmosphere (1 atm) (full  $\text{H}_2$  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/ $\text{H}_2\text{O}$  and 0.5 mL of concentrated HCl. The solution was lyophilized overnight to afford the acid **15j** (11 mg, 18 %) as a white solid.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{19}\text{H}_{14}\text{ClN}_3\text{O}_3$  requires 367, found 322  $[\text{M-H-CO}_2]^-$ , 366  $[\text{M-H}]^-$ .

**2-(6-chloro-9-(thiazole-4-carbonyl)-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 6M HCl (5 mL). The solution was stirred for 5 days at room temperature. EtOAc and  $\text{H}_2\text{O}$  were then added. After separation, the organic phase was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The residue was purified by preparative HPLC to furnish acid **15k** (18 mg, 16 %) as a white solid.  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{19}\text{H}_{13}\text{ClN}_2\text{O}_3\text{S}$  requires 384, found 339  $[\text{M-H-CO}_2]^-$ .

**Separation of enantiomers**—Compounds **1**, **15c** and **15i** were subjected to enantiomeric separation by chiral HPLC. For carprofen **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.<sup>42</sup> 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 hydrolysed with 6M 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.

**(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);  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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)  $\text{C}_{15}\text{H}_{12}\text{ClNO}_2$  requires 273, found 272  $[\text{M-H}]^-$ ; retention time on analytical chiral HPLC: 8.295 min.

**(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);  $^1\text{H NMR}$  (400 MHz,  $\text{DMSO-}d_6$ )  $\delta$  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) C<sub>15</sub>H<sub>12</sub>ClNO<sub>2</sub> requires 273, found 272 [M-H]<sup>-</sup>; retention time on analytical chiral HPLC: 10.173 min.

**(2S)-(6-chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)propanoic acid (+)-15c**—  
[α]<sub>D</sub> = +28.42 (c = 0.1, EtOAc), ee > 99.5 % (detector UV 240 nm); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>22</sub>H<sub>15</sub>Cl<sub>2</sub>NO<sub>3</sub> requires 411, found 366 [M-H-CO<sub>2</sub>]<sup>-</sup>; retention time on analytical chiral HPLC: 16.834 min.

**(2R)-(6-chloro-9-(4-chlorobenzoyl)-9H-carbazol-2-yl)propanoic acid (-)-15c**—  
[α]<sub>D</sub> = -28.72 (c = 0.1, EtOAc), ee > 99.5 % (detector UV 240 nm); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>22</sub>H<sub>15</sub>Cl<sub>2</sub>NO<sub>3</sub> requires 411, found 366 [M-H-CO<sub>2</sub>]<sup>-</sup>; retention time on analytical chiral HPLC: 23.442 min.

**(2S)-(6-chloro-9-(oxazole-4-carbonyl)-9H-carbazol-2-yl)propanoic acid (+)-15i**—  
[α]<sub>D</sub> = +39.85 (c = 0.1, EtOAc), ee > 99.5 % (detector UV 240 nm); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>19</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S requires 384, found 323 [M-H-CO<sub>2</sub>]<sup>-</sup>; retention time on analytical chiral HPLC: 24.425 min.

**(2R)-(6-chloro-9-(oxazole-4-carbonyl)-9H-carbazol-2-yl)propanoic acid (-)-15i**—  
[α]<sub>D</sub> = -39.38 (c = 0.1, EtOAc), ee > 99.5 % (detector UV 240 nm); <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) 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) C<sub>19</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub>S requires 384, found 323 [M-H-CO<sub>2</sub>]<sup>-</sup>; retention time on analytical chiral HPLC: 37.257 min.

**In vitro assays**—FAAH activity was measured by incubating for 30 minutes at 37°C [<sup>3</sup>H] anandamide (1 μM cold AEA and 0.6 nM (1 mCi/mL) [<sup>3</sup>H]-AEA (Arachidonyl-[1-<sup>3</sup>H] ethanolamine, Specific activity 60 Ci/mmol; American radiolabel Chemicals, Inc. MO, USA) in the presence of 50 μg protein/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<sub>3</sub>/MeOH. The aqueous phase was counted by liquid scintillation (Microbeta2 Lumijet, Perkin Elmer Inc., MA-USA; adapted from Kathuria *et al*, 2003). Inhibitors were pre-incubated with the enzyme preparation at the appropriate concentration for 10 minutes prior to substrate addition.

COX activity was measured using a commercial enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA). The manufacturer protocol was followed except for the substrate concentration. Briefly, inhibitors were pre-incubated 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 minutes at 37 °C. The reaction was stopped with hydrochloric acid and COX-derived PGH<sub>2</sub> was then converted to PGF<sub>2</sub>α with SnCl<sub>2</sub>. The PGF<sub>2</sub>α product is then quantified *via* enzyme immunoassay (EIA) using a PG-specific antibody and competing with a PG-acetylcholinesterase conjugate. Absorbance is measured at 412 nM with a Tecan Infinite M200 plate reader (Tecan Group Ltd., CH) and data processed according to manufacturer's instructions.



## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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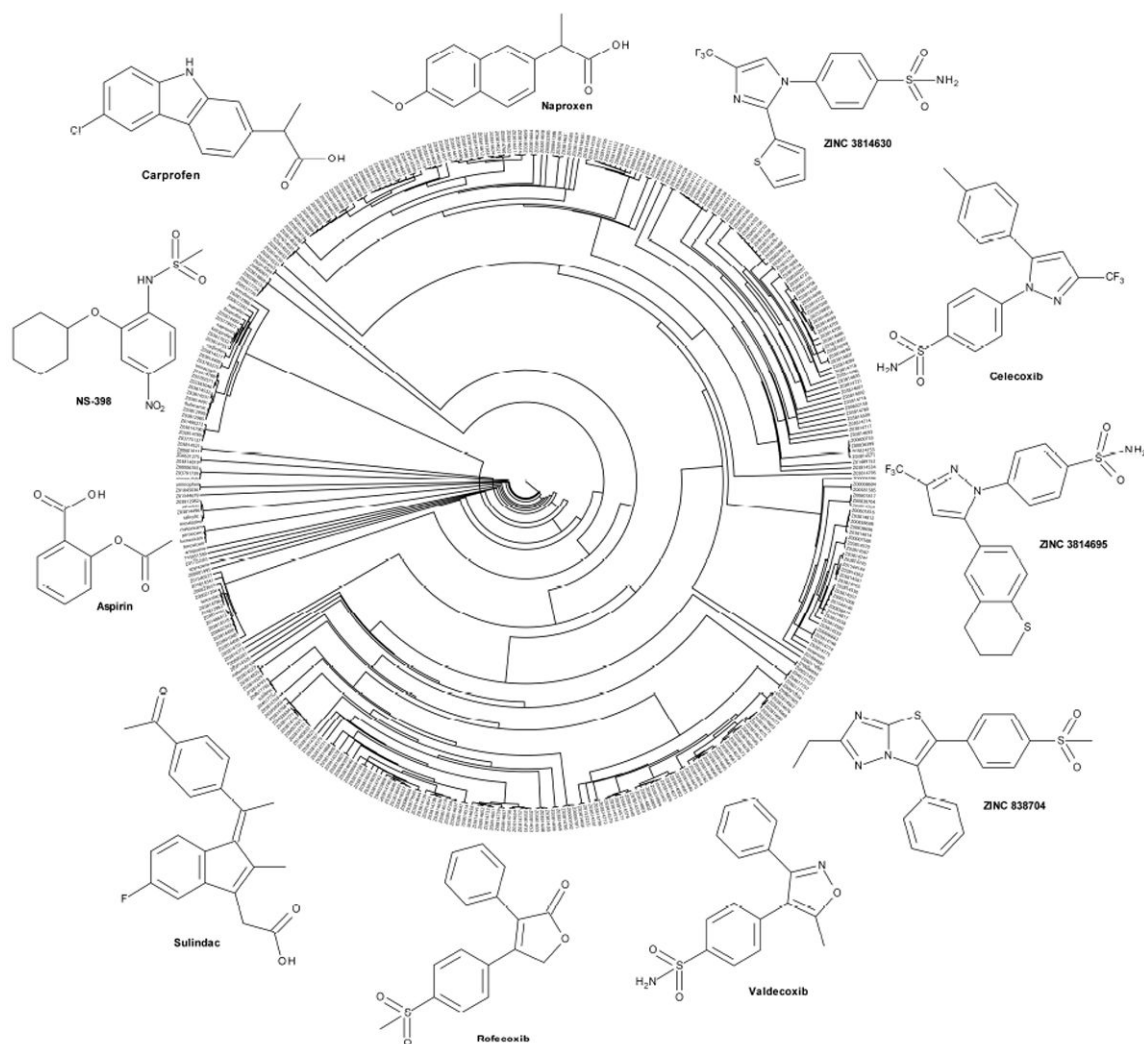
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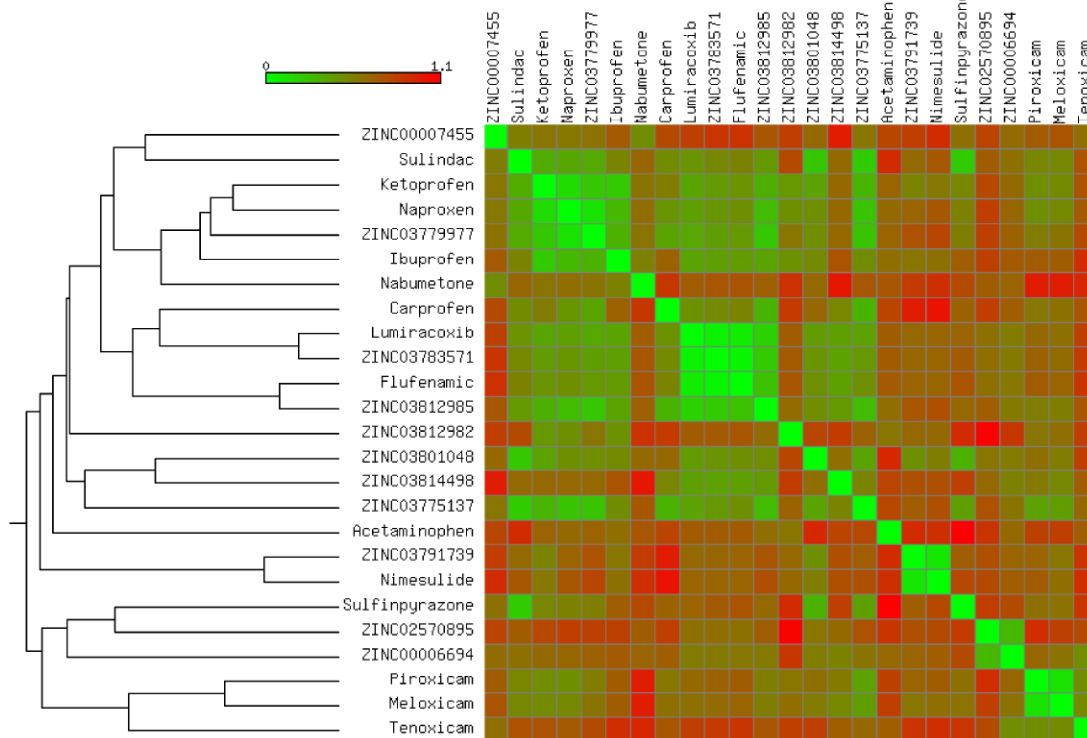
## Nonstandard abbreviations

<b>FAAH</b>	Fatty acid amide hydrolase
<b>PGE2</b>	prostaglandin E2
<b>DMAP</b>	4-Dimethylaminopyridine
<b>POX</b>	peroxidase activity



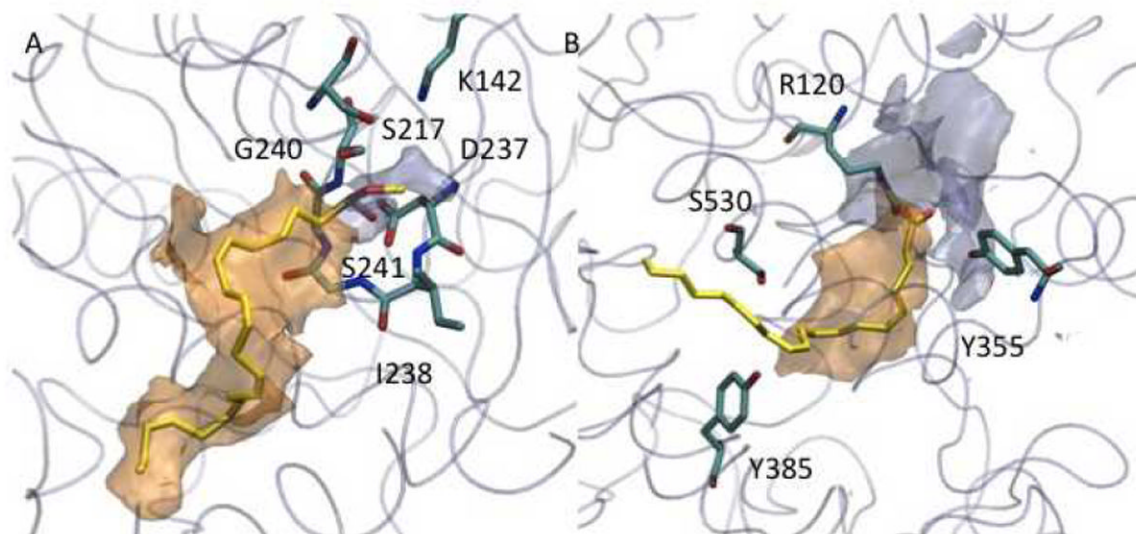
**Figure 1.** Circular tree based on pairwise Tanimoto distances between Daylight fingerprints of 382 diverse known COXs inhibitors. To help in the interpretation, only selected molecules, belonging to different clusters, are depicted in proximity of their positions in the tree to highlight the structural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



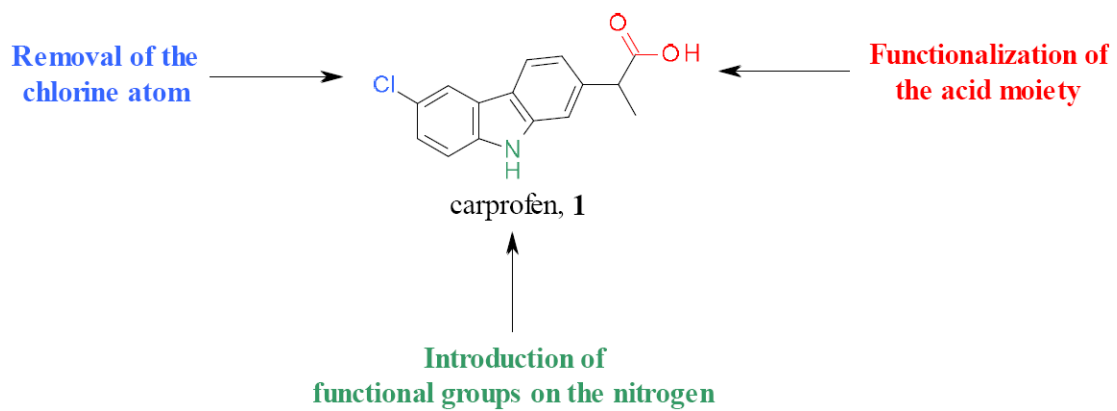


**Figure 2.**

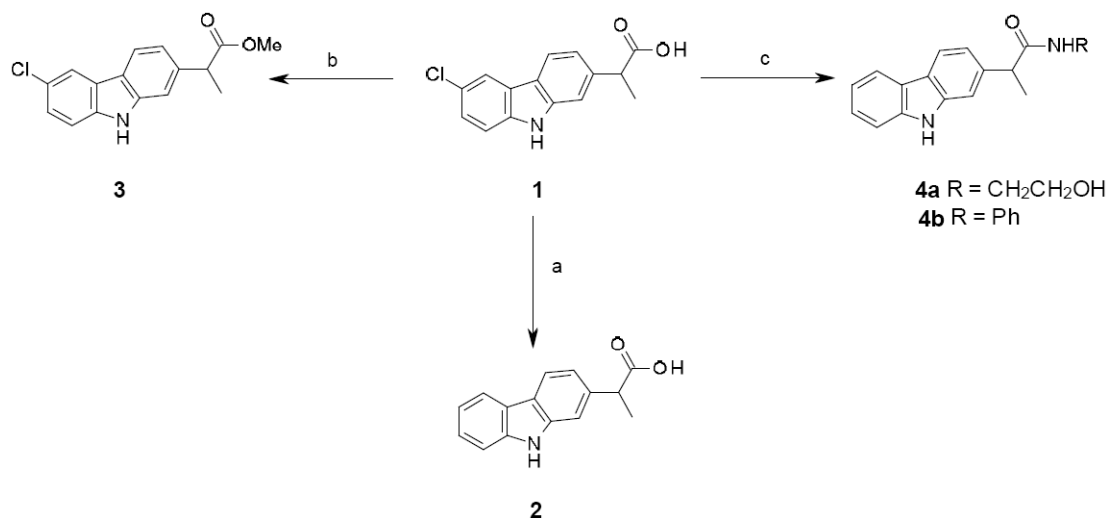
Tree based on the pairwise Tanimoto-fingerprint distances between the 25 COX inhibitors tested in the present study. The heat map highlights the distances calculated in the first 5 principal components space (% variance explained > 90%) originating from 10 physico-chemical descriptors (i.e. net charge, MW, LogP, LogS, HBD, HBA, PSA, no. of atoms, no. of rings and no. of rotatable bonds).



**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 transparent cyan tube. Substrates are methyl arachidonyl fluorophosphonate in FAAH and arachidonic acid in COX-2.

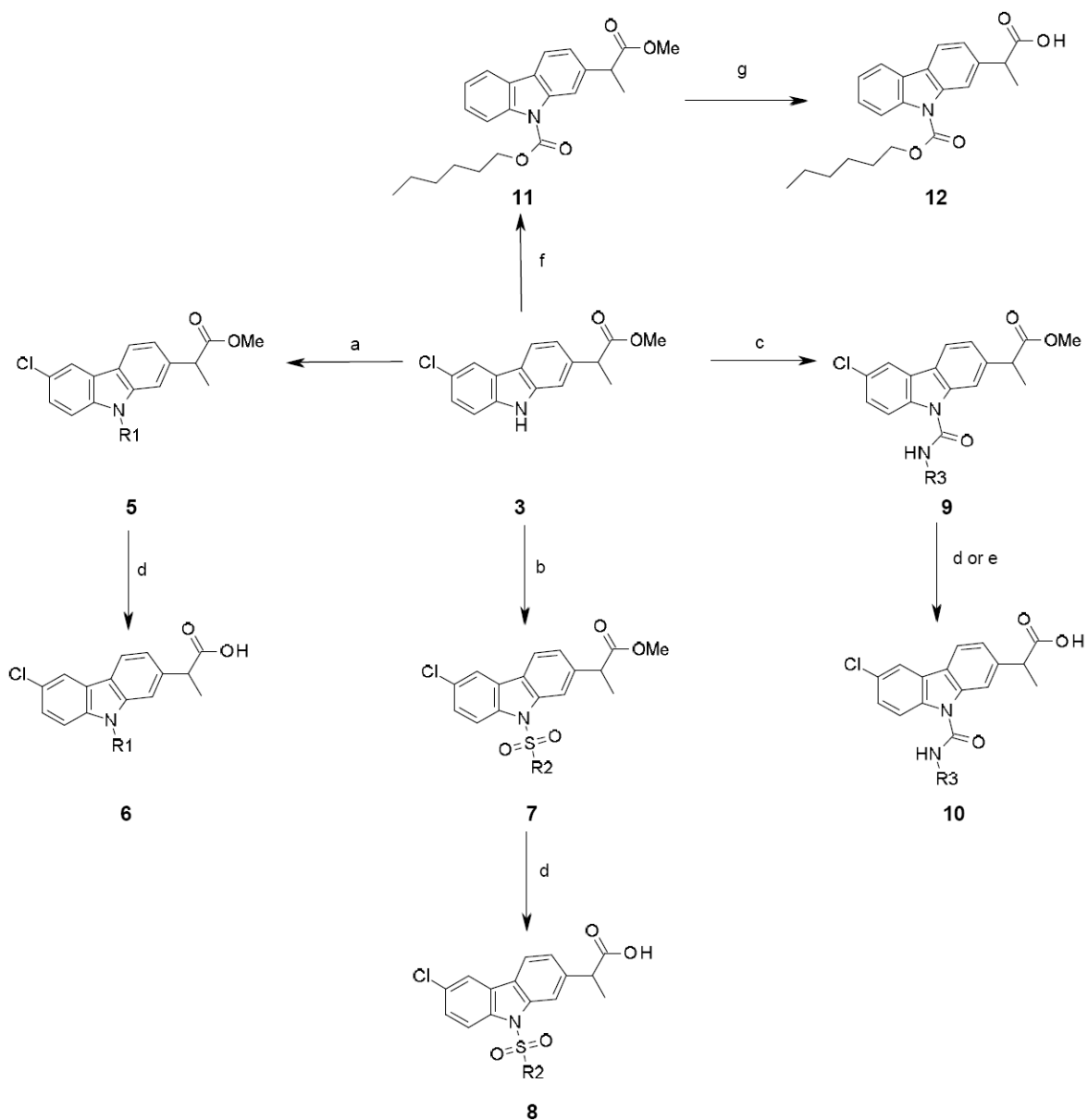


**Scheme 1.**  
Planned chemical variations of carprofen, **1**

**Scheme 2.**

Synthesis of *des*-chlorinated compound **2**, ester **3** and amides **4**<sup>a</sup>

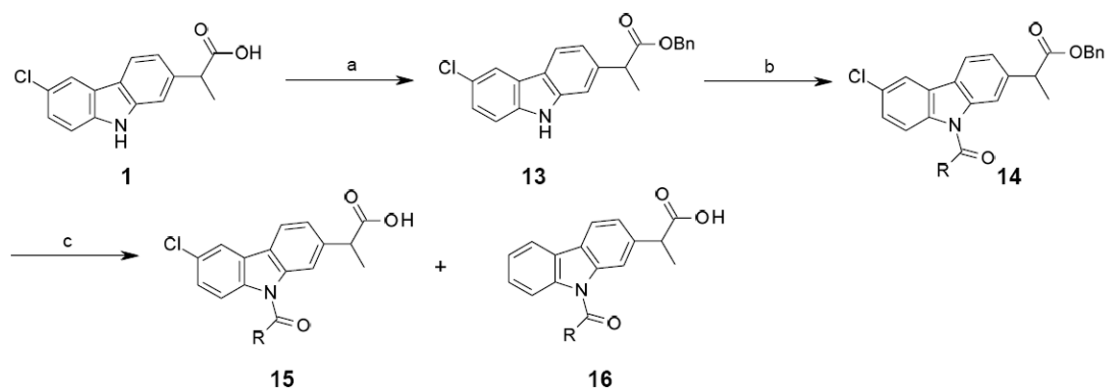
<sup>a</sup> Reagents and conditions: (a) H-Cube, H<sub>2</sub>, 10 % Pd/C, EtOH, EtOAc, 60 °C, 40 %; (b) MeOH, H<sub>2</sub>SO<sub>4</sub>, rt, 12 h, quant; (c) CDI, pyridine, 60 °C, 12 h, 85-86 %.

**Scheme 3.**

Synthesis of compounds **6**, **8**, **10** and **12**<sup>a</sup>

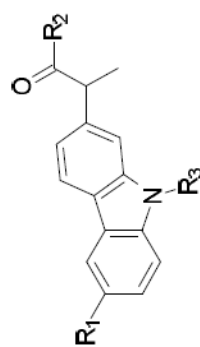
<sup>a</sup>Reagents and conditions: (a)  $R_1-X$ ,  $Cs_2CO_3$ , MeCN, reflux, 12 h, 32-99 %; (b)  $R_2-SO_2Cl$ ,  $Et_3N$ , DMAP, THF, reflux, 5 h or 100 °C, 3 h, MW, 35-78 %; (c)  $R_3-NCO$ ,  $Et_3N$ , DMAP, THF, 100 °C, MW, 10 h, 51-81 %; (d) LiOH, MeOH, THF,  $H_2O$ , 12 h, 21-85%; (e) 6M HCl, THF, rt, 5 days, 80 %; (f) hexyl chloroformate,  $Et_3N$ , DMAP, THF, 100 °C, 3 h, MW, 85 %; (g) 6M HCl, THF, rt, 3 days, 55 %.



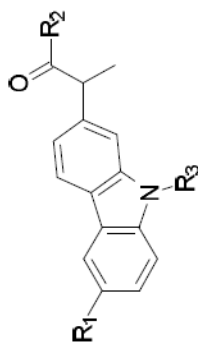
**Scheme 4.**Synthesis of compounds **15<sup>a</sup>**

<sup>a</sup>Reagents and conditions: (a) BnBr, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 3 h, 84 %; (b) Acyl chloride, DMAP, Et<sub>3</sub>N, MeCN, rt, 2 h, 76-97 %; (c) H-Cube, H<sub>2</sub>, 1 or 5 % Pd/C, rt, THF, 15-42 %.

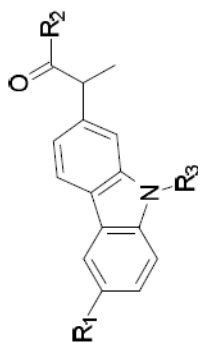
Table 1

FAAH-1, COX-1 and COX-2 activities of carprofen derivatives<sup>a</sup>

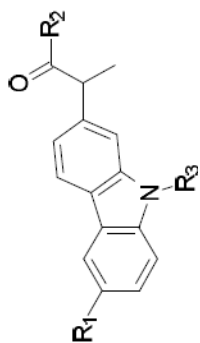
Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
				FAAH	COX-1	COX-2
1	Cl	OH	H	78.6±19.7	22.3±6.6	3.9±1.0
2	H	OH	H	4.6±0.3	/	/
3	Cl	OCH <sub>3</sub>	H	22.1±2.4	/	/
4a	Cl	HOCH <sub>2</sub> CH <sub>2</sub> NH	H	/	/	/
4b	Cl	C <sub>6</sub> H <sub>5</sub> NH	H	2.9±0.2	/	/
6a	Cl	OH	CH <sub>3</sub>	15.5±2.5	/	/
6b	Cl	OH		23.3±2.5	/	/
6c	Cl	OH		33.4±15.7	/	/
6d	Cl	OH		10.2±0.9	/	/
6e	Cl	OH		8.4±2.4	/	/



Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
				FAAH	COX-1	COX-2
<b>6f</b>	Cl	OH		32.5±7.1	/	/
<b>6g</b>	Cl	OH		/	/	/
<b>8a</b>	Cl	OH		50.3±2.3	/	/
<b>8b</b>	Cl	OH		4.8±4.5	/	/
<b>10a</b>	Cl	OH		3.2±1.7	/	/
<b>10b</b>	Cl	OH		/	/	/



Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
				FAAH	COX-1	COX-2
<b>10c</b>	Cl	OH		/	/	/
<b>10d</b>	Cl	OH		26.8±8.1	15.4±8.8	/
<b>10e</b>	Cl	OH		102.0±6.1	41.3±6.9	/
<b>10f</b>	Cl	OH		/	/	/
<b>10g</b>	Cl	OH		/	/	/
<b>12</b>	Cl	OH		31.6±0.8	17.2±1.9	/



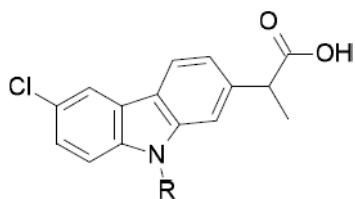
Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
				FAAH	COX-1	COX-2
<b>15a</b>	Cl	OH		/	/	/
<b>15b</b>	Cl	OH		25.4±1.9	/	/
<b>15c</b>	Cl	OH		22.0±4.2	74.3±28.0	72.3±28.0

<sup>a</sup> Values are means of 3 experiments performed in duplicate;

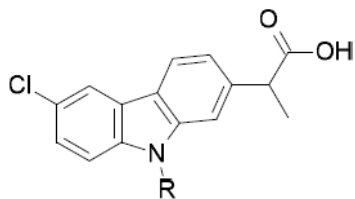
<sup>b</sup> / : IC<sub>50</sub> > 100μM



Table 2

SAR exploration around **15c**<sup>a</sup>

Compd	R	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
		FAAH	COX-1	COX-2
15c		22.0±4.2	74.3±28.0	72.3±28.0
15d		30.9±10.6	/	/
15e		10.6±2.6	/	/
15f		20.1±2.6	/	/
15g		59.6±15.5	/	/
15h		/	/	/



Compd	R	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
		FAAH	COX-1	COX-2
15i		84.8±10.6	30.0±12.1	27.8±9.7
15j		5.6±2.9	12.8±8.9	/
15k		/	/	/

<sup>a</sup>Values are means of 3 experiments performed in duplicate;

<sup>b</sup>/: IC<sub>50</sub> >100μM

**Table 3**FAAH, COX-1 and COX-2 activities of single enantiomers of compounds **1**, **15c** and **15i** <sup>a</sup>

Compd	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
	FAAH	COX-1	COX-2
(±)- <b>1</b>	78.6±19.7	22.3±6.6	3.9±1.0
<i>S</i> -(+)- <b>1</b>	64.2±3.6	5.6±0.1	5.3±3.0
<i>R</i> -(-)- <b>1</b>	/	/	/
(±)- <b>15c</b>	22.0±4.2	74.3±28.0	72.3±28.0
<i>S</i> -(+)- <b>15c</b>	/	45.0±0.3	46.5±4.3
<i>R</i> -(-)- <b>15c</b>	14.9±1.6	/	/
(±)- <b>15i</b>	84.8±10.6	30.0±12.1	27.8±9.7
<i>S</i> -(+)- <b>15i</b>	/	4.1±2.8	2.5±1.4
<i>R</i> -(-)- <b>15i</b>	53.2±22.6	/	/

<sup>a</sup>Values are means of 3 experiments performed in duplicate;<sup>b</sup>/: IC<sub>50</sub> >100μM