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Mastering tricyclic ring systems for desirable functional cannabinoid activity

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Abstract

There is growing interest in using cannabinoid receptor 2 (CB2) agonists for the treatment of neuropathic pain and other indications. In continuation of our ongoing program aiming for the development of new small molecule cannabinoid ligands, we have synthesized a novel series of carbazole and γ -carboline derivatives. The affinities of the newly synthesized compounds were determined by a competitive radioligand displacement assay for human CB2 cannabinoid receptor and rat CB1 cannabinoid receptor. Functional activity and selectivity at human CB1 and CB2 receptors were characterized using receptor internalization and [³⁵S]GTP- γ -S assays. The structure-activity relationship and optimization studies of the carbazole series have led to the discovery of a non-selective CB1 and CB2 agonist, compound **4**. Our subsequent research efforts to increase CB2 selectivity of this lead compound have led to the discovery of CB2 selective compound **64**, which robustly internalized CB2 receptors. Compound **64** had potent inhibitory effects on pain hypersensitivity in a rat model of neuropathic pain. Other potent and CB2 receptor-selective compounds, including compounds **63** and **68**, and a selective CB1 agonist, compound **74** were also discovered. In addition, we identified the CB2 ligand **35** which failed to promote CB2 receptor internalization and inhibited compound CP55,940-induced CB2 internalization despite a high CB2 receptor affinity. The present study provides novel tricyclic series as a starting point for further investigations of CB2 pharmacology and pain treatment.

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Keywords

Cannabinoid; CB2; GPCR; neuropathic pain; carbazole; CB1

1. Introduction

Two cannabinoid receptors, CB1 and CB2, have been characterized and cloned[1, 2]. The CB1 receptor is found predominantly in the brain[3]. Impairment of cognitive functions and psychoactivity induced by cannabinoids ligands are mediated by CB1 receptors activation[4]. Selective activation of CB2 receptors has been proposed as a strategy to curtail the negative central side effects seen with nonselective CB1/CB2 agonists. Several CB2-selective agonists have been described previously[5–11]. Although multiple preclinical studies suggest that the CB2 receptor is a viable target to decrease both acute and neuropathic pain responses[12, 13], synthetic CB2 agonists have not advanced through clinical trials. In part, this is due to a lack of a thorough understanding of CB2-mediated analgesic mechanisms[14]. CB2 modulation is also implicated in immunomodulation and neuroprotection but the functional profile of the CB2 ligands inducing these effects has not been clearly defined[15]. Activation of CB2 receptor induces its coupling to the Gi/o class of G proteins. The dissociation of the α and $\beta\gamma$ subunits resulting from the CB2 activation can influence multiple effector systems including adenylyl cyclase, p42/44 MAPK (ERK1/2) or ion channels[16]. Currently, the diversity of cannabinoid agonists is very broad and continues to expand rapidly (figure 1). In our previous communications, the SARs of potent and selective CB2- receptor agonists[17] and antagonists[6] based on the isatin scaffold were described (MDA19 in Figure 1).

Although the isatin core was identified as a good scaffold for designing CB2-selective cannabinoid agonists, we hypothesized that the intramolecular hydrogen bond pattern in the isatin scaffold can be replaced with a covalent C-C bond. Also considering the high pharmacological potential of carbazole-based natural products[18], we focused our attention on this scaffold for use of studying CB1/CB2 receptor pharmacology. Thus, we first identified compound **4** as a potent non-selective CB1/CB2 agonist[19]. We recently reported the cannabinoid and T-type calcium channel activities for three compounds from this novel series of cannabinoid ligands[7]. In this article, we optimized the CB2 selectivity of this series. We also explored the effect on CB2 activity of the introduction of quaternary ammonium moieties or introduction of heteroatoms increasing the polar surface area to help future identification of peripherally-restricted CB2 agonists in our tricyclic series. This research effort led to the discovery of a selective CB2 agonists with a polar surface area $> 70 \text{ \AA}^2$ and expected to have a low blood-brain barrier (BBB) permeability. Functional activities of our most active compounds were determined using [^{35}S]GTP- γ -S assays and by characterizing their ability to internalize human CB1 and CB2 receptors. The ability of these compounds to interact with the orthosteric site on human CB2 receptor was assessed by determining their ability to block CP55940-induced hCB2 internalization. Moreover, the binding mode prediction through ligand-steered modeling highlighted a potential H-bond interaction between the alkylsulfonamide moiety in the N-1 position bore by compound **64** and N7.45 of the CB2 receptor. Similarly to what was previously observed within the isatin series, the presence of the OMe fragment in the carbazole scaffold turned out to be a key substituent responsible for the agonist-to-antagonist functionality switch.

2. Results and discussion

2.1. Chemistry

The pathway of achieving the synthesis of the first series of compounds (**4-11** and **14-23**) is outlined in Scheme 1. We found that desirable analogs **4-11** can be conveniently prepared from commercially available carbazole via consecutive substitution with *n*-pentyl bromide under alkaline conditions followed by electrophilic formylation using standard Vilsmeier–Haack conditions, oxidation, and, finally, amidation under standard peptide coupling conditions. Later, a more convenient method was developed for the synthesis of compound **4** that utilized a direct Friedel–Crafts reaction using piperidinecarbonyl chloride. While our conditions for the Friedel–Crafts reaction (compounds **4**, **18**, and **19**) were not expected to be optimal, this strategy did provide a rapid access to the desired compounds. Compound **19** was subjected to nucleophilic attack by the corresponding amines to give analogs **20-22**. Deprotection of the Boc protecting group in compounds **12** and **13** furnished compounds **14-15**. Analog **16** and **17** were prepared by coupling of corresponding bromomethylpyridyl derivatives and acid **3** in the presence of TBAF under basic conditions [20]. The tertiary base **9** was converted to the quaternary form **23** by treatment with methyl iodide in diethyl ether at room temperature.

Next, we focused on modifications (Scheme 2) that led to a series of compounds in which the original 3-carbonyl group in the carbazole framework was either derivatized or entirely eliminated. Compounds **24-26**, **28** and **30** were synthesized following protocols as outlined in Scheme 2. Nitrile **24** was prepared by a one-pot solvent-free procedure from aldehyde **2** and hydroxylamine. Thioamide **25** was prepared by heating amide **4** with Lawesson's reagent under microwave conditions. Tertiary amine **26** was obtained upon treatment of **4** with LAH. The preparation of amines **28** and **30** was achieved by palladium-catalyzed coupling in analogy to known literature methods (e.g.[21]).

The pyrido[3,4-*b*]indole-based analogs **33-35** were prepared as depicted in Scheme 3. Commercially available ethyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate was alkylated with *n*-pentyl bromide under microwave conditions. Hydrolysis of ester **31** gave the corresponding carboxylic acid **31**. Activation of acid **32**, followed by coupling with the corresponding amines provided the corresponding target carboxamides **33-35**.

A series of *N*-substituted analogues were prepared according to Scheme 4. In an analogous fashion as previously described for compound **4**, *N*-alkylations of carbazole (compounds **36-39**) followed by Friedel–Crafts reaction using piperidinecarbonyl chloride onto the resultant substrates led to analogs **40-42**. *N*-alkyl derivatives **43-44** were prepared according to the literature[22]. Intermediate **44** was prepared from carbazole and ethylene oxide under basic conditions. Conversion of alkyl carbazoles **37**, **44**, and **45** to the corresponding aldehydes **46-49** was achieved using standard Vilsmeier–Haack conditions as described above. Vilsmeier–Haack reaction allowed simultaneous installation of an aldehyde group into the aromatic core and a halogen atom into the *N*-alkyl chain. Similarly, the obtained aldehydes were then oxidized to the corresponding carboxylic acids **50-53**. After activation, these acids were used for coupling with the corresponding amines under standard peptide coupling conditions to furnish compounds **54-57**. The *N*-alkylated chloride derivatives **56** and **57** were converted to the corresponding iodides **58** and **59**. Taking advantage of the ease of nucleophilic displacement of the iodide substituent, compounds **60-64** were obtained by base-mediated aminolysis of compounds **58** and **59**. Although some of the targets from this series could still be prepared starting from carbazole, we realized that it would more expedient to achieve the same goal by using Buchwald–Hartwig amination and subsequent palladium-mediated oxidative cyclization of the diarylamines [23]. Following this strategy,

the diaryl amine **65** was formed and then oxidized with Pd(OAc)₂ to give the carbazole ester **66**. Subsequent ester hydrolysis of **66** delivered the corresponding acid which was amidated with piperidine to produce the generic compound **67**. Alkylation of carbazole **67** afforded the target compounds **68-76**. Deprotection of the Boc group within **72** afforded compound **73** after aqueous work-up with sodium hydroxide.

Following a procedure analogous to that of Scheme 4 for compound **67**, compounds **81**, **84**, and **85** were prepared as depicted in Scheme 5. Alternatively, further modification of the carboxamide part of analog **55** was enabled starting from carboxylic acid **51**, which was subjected to amide coupling conditions to afford the desired amides **82** and **83**.

2.2. Binding affinities for cannabinoid receptors and modeling: structure-activity relationship

We previously identified a series of isatin acylhydrazone CB2 modulators[6, 17]. These compounds showed potent CB2 agonist or antagonist activities. The lead compound among the isatin series possessed potent antiallodynic effects in a rat model of neuropathic pain without affecting the rat locomotor activity[13] at the therapeutic dose. We next turned our attention to substituting the isatin acylhydrazone core with a carbazole scaffold, which is devoid of the potential hydrazone bond isomerization and would have improved potency due to its rigid structural template that may ensure the precise spatial orientation of important functional groups.

Starting with the lead compound **4** we carried out structural modifications in order to expand the SARs and optimize binding affinity, selectivity, and functional activity associated with the CB2 receptor. The CB1 and CB2 receptors binding affinities were determined by performing [³H]CP55,940 radioligand competition binding experiments (Table 1, 2, 3, 4 and 5). Previous studies have shown that binding affinities of CB1 ligands were in the same range for human and rat CB1 receptors[12, 24].

The structural model of **4** complexed with the CB2 receptor is shown in Fig. 2. The ligand occupies a cavity defined by helices 3, 5, 6 and 7, with the alkyl chain buried within the binding site, and the piperidine moiety facing the extracellular side. In agreement with this predicted binding mode, deletion of the chain born by the endocyclic nitrogen in **67** was detrimental to its binding. Replacement of the piperidine ring by a nitrile moiety in **24** or a methyl ester moiety in **31** had a strong negative effect on CB2 affinity, probably due to the lack of ligand-receptor van der Waals contacts. In order to further improve the physicochemical properties and metabolic stability of compound **4**, we decided to incorporate polar groups at position 4 of the piperidine ring[26]. However, such a chemical modification did not produce significant improvement in affinity towards the CB2 receptor for analogues **6** and **8**. Alternatively, replacing the piperidine ring by the more polar N-methylpiperazine ring (compound **9**), decreased affinity at CB1 but increased toward CB2, resulting in a 3-fold improvement of CB2 selectivity compared to the piperidine analogue **4**. It is notable that the removal of the methyl moiety (compound **15**) from the N-methylpiperazine analogue, dramatically decreased the CB2 binding affinity indicating that a hydrophobic moiety in this part of the molecule may be essential for the CB2 affinity. In contrast, the CB1 affinity in this case decreased by only 4-fold. Next, the quaternary ammonium derivative **23** was prepared in order to probe electrostatic interactions with CB2 and investigate if this strategy can be used to design CB2 agonists that do not cross the blood-brain barrier. Unfortunately, compound **23** turned out to be devoid of CB1 or CB2 affinity. This result lends further support to our view that lipophilicity is a required feature on the carboxamide part of the molecule. Since there was compelling evidence that a lipophilic moiety is required for a good CB2 affinity, we decided to add a methylene moiety

or a nitrogen spacer in between the carbonyl and the lipophilic piperidine moiety to test whether the biological profile can be further improved. However, as compared to the piperidine analogue **4**, the N-aminopiperidine derivative **7**, the N-methylpiperidine derivative **20** and the methylpiperidine derivative **14** did not show relevant CB1 or CB2 affinities, thus suggesting a scarce adaptability of the lipophilic pocket in the CB2 binding site. One can notice that among common features present in these three compounds is not only a longer substituent, but also a tertiary amine offering a site of N-cation interactions. Therefore, it may be speculated that perhaps either the increased basicity of the tertiary amine or the subsequent cation interactions are responsible for destabilizing the complex. The thiomorpholine 1,1-dioxide analogue **21** exhibited moderate affinities for both CB1 and CB2. Our attempts to increase the conformational flexibility in the carboxamide region by opening the piperidine cycle, as exemplified by compound **5** slightly increased CB2 affinity and the selectivity toward CB2. Replacement of the piperidine by the planar phenyl ring, as in compound **18**, resulted in a slight decrease of CB1 affinity, while the CB2 affinity was in the range of the parent compound. These observations support the binding mode proposed for **4** (Figure 2). On the other hand, an increase of lipophilicity in compound **10**, resulted in an improvement in CB2 selectivity. In this case the adamantyl group is probably too bulky to fit properly in the CB1 receptor-binding pocket. One of the unique benefits of “soft drugs” such as esters for treating inflammatory disorders lies in the susceptibility of their ester functionality to hydrolysis, which can limit their CNS penetration. Therefore, such molecules should lack the undesired CNS-induced side effects associated with CB1 receptor activation. The pyridine ester analogues **16** and **17** were synthesized to build aqueous solubility required for bioavailability *via* the hydrolysable ester bonds. Surprisingly, both compounds exhibited higher affinity at the CB1 rather than at the CB2 receptor. This decrease in CB2 affinity is in good agreement with what is reported above regarding the longer substituents. Further exploration of the SARs around the carbazole series led us to examine the impact of the amide toward CB2 affinity. Replacement of the carbonyl (compound **4**) by a methylene in compound **26** resulted in a decrease of CB2 affinity while the CB1 affinity was in the same range, what could be explained in terms of the lost hydrogen-bond with the OH group of S7.39 (cf. Figure 2). On the other hand, the replacement of the carbonyl by the thiocarbonyl in **25** resulted in the CB2 affinity being restored. Replacement of amide bonds by thio-amide bonds have been shown to destabilize hydrogen bond by the higher steric demands imposed by the larger sulfur atom, which leads to non-optimal angles required to form hydrogen bonding[27]. In addition, the sulfur atom has lower electronegativity compared to oxygen atom. However, C=S bond in **25** may induce the required conformation and with the correct position of the piperidine ring needed for high CB2 affinity[28]. Nevertheless, thioamide derivatives cannot be considered as viable alternatives to design potent CB2 agonists since it was shown that thioamides behave as amide prodrugs *in vivo*. As expected, the piperazine analogues **28** and **30** did not exhibit potent CB2 affinity even though the CB1 affinity was slightly lower for **28** compared **9**. Introduction of an extra nitrogen atom in the carbazole scaffold was expected to impact both water solubility and positioning of the lipophilic moiety attached to the carbonyl. As a result, CB2 affinity was slightly increased for the carboline analogue **33** when compared to the respective carbazole analogue. For the former, the CB1 affinity was in the same range as for its carbazole counterpart **4**. Notably, the CB2 affinity was substantially higher for the N-aminopiperidine carboline derivative **34** as compared to the carbazole derivative **7**. This higher affinity could potentially result from a hydrogen bond between the secondary amino group and the nitrogen on the carboline scaffold, thus providing some critical assistance in accommodating the respective lipophilic moiety in the CB2 binding site. Introduction of the neopentyl moiety in **35** resulted in increased affinity at both CB1 and CB2 but the selectivity toward CB2 receptor was rather low (*ca.* 1.65).

In the course of our initial exploratory work on the structure–activity relationship for this novel series of CB2 modulators, we decided to determine the optimal chain length attached to the carbazole's endocyclic nitrogen (compounds **40** to **54**). Among the linear N-1 alkyl chains, a pentyl chain seemed to be the most optimal for occupying the CB2 lipophilic cavity, because systematically increasing and decreasing the length from *n*-pentyl in **4** negatively impacted the respective CB2 affinities. Introduction of halogen atoms was shown to increase the affinity of small molecules toward proteins even though halogen bond interactions are significantly weaker than hydrogen bond interactions. Previous results from literature suggest that interactions between C-Cl moieties and carbonyl groups or other H-bond acceptors increase affinity of corresponding ligands to biological targets[29]. Introduction of a chlorine atom into the ethyl or propyl chain of **56** and **57** did not increase CB2 affinity. The longer chloropropyl chain in **57** mimicked the pentyl chain in **4**, while the use of a shorter one in **56** suffered a dramatic loss of CB2 affinity. Hydrogen bond interactions were shown to provide specificity to the process of receptor recognition although not always did they add much of free binding energy[30]. Since we achieved only modest improvements in terms of CB2 selectivity, we next sought to expand our library by targeting potential hydrogen bond interactions between the chain carried by the endocyclic nitrogen and CB2 receptor. First, we turned our attention on to alkyl ethers, despite the fact that ethers are known to be weak hydrogen bond acceptors. However, an interesting feature of ethers in this regard is that they exhibit a somewhat higher tolerance for angular requirement between the hydrogen-donor and acceptor[30]. In order to explore the SARs within this context, we synthesized acyclic (compound **68**) and cyclic (compound **69**) ethers. The cyclic ethers are known to be stronger hydrogen bond acceptors than the acyclic ones[30]. Consistent with our predictions (Fig. 2), **69** demonstrated higher CB2 affinity as compared to **4** although the CB2 selectivity remained poor (Fig. 2). A replacement of the 4-methyltetrahydropyran fragment by the 4-methylpyridine moiety as exemplified by compound **74** resulted in a 4-fold improvement in CB2 selectivity, while the respective CB2 affinities were similar. The binding mode of **74** is shown in Fig. 3, exhibiting a similar pose when compared to **4** with hydrogen bonding occurring between the amide carbonyl of **74** and S7.39. In this case, the only difference is that an additional hydrogen bond is present between the nitrogen of the 4-methylpyridine and the side chain of W6.48. Consistent with this model, the 2- and 3-methylpyridine analogues (compounds **75** and **76**) exhibited low CB2 affinities. In contrast replacement of the pentyl chain by the acyclic ether in compound **68** resulted in a significant increase of selectivity due to a specific decrease CB1 affinity. Acyclic esters functionality such as in compound **70** resulted in a dramatic improvement in selectivity due to significantly lower affinity of this compound at the CB1 receptor. Interestingly, this effect was observed for both the ester analogue and the dimethyl amine analogue **71**. A significant loss of CB2 binding affinity in these cases may be attributed to three obstacles such as higher degree of angular preferences, higher steric demands, or difference in electronic properties. In comparison, the ethyl ester counterpart **55**, which has the ester functionality closer to the carbazole scaffold did not exhibit significant affinity at CB1, and its affinity at CB2 was not promising either. In part, this might be explained by poor ability of **55** to promote hydrogen bonding with the corresponding receptors. When the nitrogen atom was free of substituents as in **73** or carried a bulky Boc moiety as in **72**, a significant loss of CB2 affinity was observed. In the former case, the charged terminal amine group was likely to destabilize the complex, which is consistent with our previous observations. Employment of alkyl chains featuring the sulfonyl functional group is typical in cases when a further increase in the PSA values of the corresponding analogs is desirable. The dual character of the weakly polar sulfonyl group which allows it to form hydrogen bonds and at the same time participate in nonpolar hydrophobic interactions through van der Waals contacts drawn our attention to the possibility of applying this strategy to improve

CB2 affinity[30, 31]. As expected, the methyl sulfonamide derivative **64** demonstrated the most specific CB2 affinity.

The binding mode of compound **64** is shown in Figure 4; the pose is very similar to that of compound **4** (Fig. 2). The first thing one can notice in this model is a weak hydrogen bond between the oxygen of the sulfonyl group and the side chain of N7.45 in addition to a hydrogen bond between the amide carbonyl of **64** and S7.39. Introducing a basic residue such as a piperidine ring into the N-aliphatic side chain resulted in the inactive compounds (**61** and **60**). A possible explanation for this might be that the charged amine in this case is buried within the potential hydrophobic binding site. In contrast, the dioxothiomorpholine counterpart **62** regained both good selectivity and CB2 affinity. A similar trend was observed for analogue **63** which offered a 30-fold selectivity over CB1. For comparative purposes, we next decided to carry out structure-activity relationship studies looking at the effects of ring substitution to determine if they would parallel those in our previous isatin series. Indeed, we observed a similar result when a methoxy group was introduced in position 6 of the carbazole scaffold resulting in compound **81** which displayed a higher CB2 affinity and was endowed with inverse agonist instead of agonist functional activity.

In the carbazole series, we also briefly explored whether it is possible to impact the spatial position of the N-alkyl chain by introducing substituents in position 4 of the piperidine ring. As shown in Table 5, both the methylpiperazine analogue **82** and the dioxothiomorpholine analogue **83** turned out to be lacking CB2 affinity. This result may be explained by the fact that the methylpiperazine and dioxothiomorpholine rings did not allow the chain containing the ester moiety to be set further down into the lipophilic pocket in order to restore the CB2 affinity within the same range of **70**. As expected (compare with **72**), the dioxothiomorpholine analogue **85** bearing a distal Boc moiety on the N-propyl chain showed no measurable activity against both the CB1 and CB2 receptors.

2.3. Cannabinoid receptors functional activity

Functional activity for compound **64** was evaluated by using a [³⁵S] guanosine-5'-triphosphate (GTP)- γ -S assay in Chinese hamster ovarian cell membrane extracts expressing recombinant hCB1 or hCB2 receptor (Table 6). In this system, agonists stimulate [³⁵S]GTP- γ -S binding, whereas antagonists have no effect and inverse agonists decrease [³⁵S]GTP- γ -S basal binding. Efficacies (Emax) for CB1 or CB2 were expressed as a percentage of the efficacy of compound CP55,940. Compound **64** behaved as a selective CB2 agonist since it did not show any agonistic or antagonistic activities at human CB1 receptors in the [³⁵S] GTP γ S assay at 1 μ M whereas the EC₅₀ value for the CB2 receptor was 7.3 nM, with an efficacy of almost 60%. Thus, compound **64** appears to be a selective CB2 agonist.

Receptor internalization and functional selectivity are important emerging pharmacological concepts that increase the complexity of the biological profiling of G protein-coupled receptor (GPCR) ligands. Recently, it has been shown that WIN55,212-2 and other aminoalkylindoles failed to promote CB2 receptor internalization, whereas compound CP55,940 robustly internalized CB2 receptors[14]. Despite these differences in term of CB2 induced-internalization, both compounds activated CB2 receptors mediated ERK 1/2 phosphorylation and recruited β -arrestin₂ to the membrane. In contrast, whereas CP55,940 inhibited voltage-gated calcium channels via CB2 receptor activation, WIN55,212-2 was ineffective on its own and antagonized the effects of CP55,940. These differences in terms of functional activity and the polypharmacology associated with cannabinoids ligands[7, 32] can possibly explain the variability observed in preclinical models of neuropathic pain and the difficulties of translating results from the preclinical models to human pain states. As a way for rapidly screening for orthosteric interactions with CB1 and CB2 receptors,

internalizations of these receptors induced by our most active compounds was determined (Table 7). In our series of compounds both CB1 and CB2 receptor internalization correlated well with the GTP γ [³⁵S] functional assays since **81**, a partial CB1 agonist and CB2 inverse agonist in the GTP γ [³⁵S] functional assays only promoted 33% of CB1 internalization and failed to internalize CB2 at 10 μ M. In contrast, **4** which was a full CB1 agonist and a partial CB2 agonist in the GTP γ [³⁵S] functional assay, promoted internalization of both hCB1 and hCB2 to similar extent. Despite high CB2 affinities, compounds **5**, **9**, **18** and **63** promoted only partial CB2 internalization. These data suggest that these compounds behave as partial agonist at CB2, at least with respect to the internalization. Similarly, **6** and **35** behaved as CB1 partial agonists as they partially induced CB1 internalization despite the high CB1 affinity. Interestingly, compound **26** failed to induce CB1 internalization despite a CB1 affinity of around 100 nM. This result may be explained by the fact that the carbonyl moiety between the carbazole scaffold and the piperidine is essential for CB1 internalization as exemplified by compound **4**, which induced CB1 internalization. Low CB1 affinity is associated with low CB1 induced internalization as illustrated by compounds **70** or **20**. The lack of CB2 internalization observed with **35** could potentially result from the hydrogen bond between the secondary amino group and the nitrogen on the carboline scaffold. This hydrogen bond may compromise the conformational flexibility of **35** required by the CB2 receptor lipophilic pocket to accommodate the neopentyl moiety essential to induce internalization. Compound **35** produced little CB1 internalization despite a good affinity, behaving as a partial CB1 agonist.

To further explore the concentration dependence of this effect, we performed complementary experiments with hCB2 cells. First, we applied a constant 10 nM compound CP55,940 with cotreatments of 3 μ M of selected compounds. As expected, the CB2 inverse agonist compound **81** reduced compound CP55,940-induced internalization in hCB2 cells (Fig. 5). Unexpectedly, compound **18** and **35** reduced by more than 50% hCB2 internalization induced by CP55,940. These compounds may behave as inverse agonists or despite their effect on internalization, they may activate CB2 receptors without inducing receptor internalization as has recently been shown for compound WIN55,212-2. A more detailed investigation into the CB2 functional activity profiles of these compounds is required to confirm these hypotheses.

2.4. In vivo studies

Peripheral nerve injury can cause clinically relevant chronic neuropathic pain. The L5 and L6 spinal nerve ligation produced long-lasting mechanical hypersensitivity (tactile allodynia) on the ipsilateral hind paw in rats. Since compound **64** exhibited a favorable CB2 selectivity profile and induced CB2 internalization, we were interested in characterizing its activity in the SNL *in vivo* model of neuropathic pain. Compound **64** administered intraperitoneally (5 – 20 mg/kg) significantly attenuated tactile allodynia in a dose-dependent manner. The higher doses (20 mg/kg and 10 mg/kg) produced a longer duration of the antiallodynic effect than that observed with the 5 mg/kg of compound **64** (Figure 6).

3. Conclusion

In this investigation, we presented a broad range of experimental data on the novel series of carbazole-based cannabinoid ligands. Within this series, sulfonamide analogue **64** was identified as a selective CB2 agonist. Our structure modeling and docking studies for compound **64** based on the ligand-steered approach highlighted a potential H-bond interaction in a burrow-like site between the alkylsulfonamide moiety at the *N*-1 position and N7.45 of the CB2 receptor. This selective CB2 ligand exhibited functional agonist activity as assessed by using [³⁵S]GTP- γ -S assay and the CB2 internalization study.

Similarly to our isatin series, the presence of a methoxy group in position 7 of the carbazole scaffold in compound **81** turned out to be a key substituent responsible for the agonist-to-antagonist functionality switch. Compound **81** exhibited [³⁵S]GTP- γ -S inverse agonist activity and inhibited compound CP55,940-induced CB2 internalization. On the basis of our results, compound **35** has a high CB2 affinity, inhibits compound CP55,940-mediated internalization, and merits further pharmacology characterization. Compound **64**, which induced CB2 internalization, attenuated tactile allodynia in a dose-dependent manner in the SNL *in vivo* model of neuropathic pain. In summary, we have identified a novel series of tricyclic CB2 selective agonists with a well-defined CB2 functional activity that can be used as a platform for the future development of specific CB2 agonists as treatments of pain. The present study also provides an additional insight into the internalization of CB2 receptors induced by CB2 agonist, which should further facilitate optimization of this novel class of tricyclic CB2 modulators for the treatment of pain.

4. Experimental Section

4.1. Synthesis

Unless otherwise stated, all reactions were carried out under a nitrogen or argon atmosphere, using commercially available reagents and solvents. Anhydrous THF and Et₂O were obtained by distillation from sodium and benzophenone followed by distillation from LAH. All other solvents are reagent grade and were used without further purification. All procedures were carried out at room temperature unless otherwise stated. Magnesium sulfate was used as the drying agent. The crude products were purified by flash chromatography using prepacked Biotage® cartridges on a Biotage® Isolera separation system. Analytical thin-layer chromatography (TLC) was performed on precoated, aluminum-backed silica gel (200 μ m thick, Sorbent Technologies, UV254). Melting points were obtained on a Start SMP3 melting point apparatus and are uncorrected. The microwave irradiation was effected using a Biotage® Initiator microwave synthesizer. High Resolution mass spectrometry (HRMS) analyses were performed on a Waters/Micromass LCT-TOF instrument. The HPLC systems used to analyze the target compounds were either: (i) Waters 2790 high-performance liquid chromatograph with an autosampler connected to a Waters 2487 dual absorbance UV detector and Waters Micromass LCT KC290 mass spectrometer or (ii) Waters Alliance e2695 high-performance liquid chromatograph with an autosampler connected to a Waters 2998 photodiode array detector and Waters Micromass LCT Premier mass spectrometer. NMR spectra were obtained either on Varian Inova-500 or Bruker Ascend™-400 instruments. Chemical shifts for ¹³C NMR spectra are recorded in parts per million using either the central peak of deuterated chloroform (77.23 ppm) or deuterated DMSO (39.51 ppm) as the internal standards. Characteristic splitting patterns due to spin-spin coupling are expressed as follows: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet. All coupling constants are measured in hertz. All synthesized piperidine-amides, except for thioamide analogs, displayed a characteristic broadening effect in their ¹³C NMR spectra for α - and β -CH₂ carbons of the piperidine ring, reflecting a restricted rotation around the peptide bond. For this reason, the corresponding chemical shifts values for these analogs were reported only when possible. 2-Hydroxypropyl- β -cyclodextrin with an average degree of molar substitution of 4.4 was purchased from CTD Holdings Inc (Alachua, FL, USA)

4.1.1. 9-Pentyl-9H-carbazole (1)

Method A: Under argon atmosphere, a solution of carbazole (2.5 g, 14.95 mmol), 1-bromopentane (2.225 mL, 17.94 mmol), and Cs₂CO₃ (7.3 g, 22.41 mmol) in DMF (10 mL) was subjected to microwave irradiation at 140°C for 1 h. The reaction mixture was cooled, diluted with EtOAc (50 mL), and filtered. The organic solvents were evaporated *in vacuo*.

The resultant dark oil was distilled under reduced pressure (bp 125°C, 2 mmHg) to afford the title compound as light yellow oil (3.169 g, 89%). ¹H NMR (500 MHz, CDCl₃) δ 8.08 (dd, *J* = 7.6, 0.8 Hz, 2H), 7.47 – 7.41 (m, 2H), 7.37 (d, *J* = 8.2 Hz, 2H), 7.21 (td, *J* = 7.5, 1.0 Hz, 2H), 4.24 (t, *J* = 7.3 Hz, 2H), 1.89 – 1.77 (m, 2H), 1.40 – 1.26 (m, 4H), 0.85 (t, *J* = 7.1 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 140.64 (C), 125.75 (CH), 123.02 (C), 120.52 (CH), 118.88 (CH), 108.84 (CH), 43.23 (CH₂), 29.62 (CH₂), 28.87 (CH₂), 22.69 (CH₂), 14.15 (CH₃).

Method B: A mixture of carbazole (10 g, 59.81 mmol), *n*-pentyl bromide (11 mL, 88.92 mmol), and finely ground NaOH (4 g, 100 mmol) in dry acetone (100 mL) was refluxed for 16 h under nitrogen. After all volatile components were removed by rotary evaporation *in vacuo*, the residue was extracted with *tert*-butyl methyl ether (150 mL). The organic phase was washed with water, brine, dried (MgSO₄), filtered, and evaporated *in vacuo*. The obtained residue was crystallized from ice-cold ethanol. Yield: 12.72 g (90%); mp 51°C. NMR spectra are identical with those by *Method A*.

4.1.2. 9-Pentyl-9H-carbazole-3-carbaldehyde (2)—The title compound was prepared according to a modified literature procedure[33]. POC₁₃ (2.6 mL, 28.40 mmol) was added, over a period of 10 min, to an ice-cooled, stirred DMF (7.43 mL, 95.96 mmol) under nitrogen. The reddish solution was allowed to stir at room temperature for 1 h. 9-Pentyl-9H-carbazole (**1**) (3.169 g, 13.35 mmol) was added over 10 min, and the obtained mixture was subjected to microwave irradiation at 100°C for 1 h. The reaction mixture was cooled and then poured into crushed ice. After warming to room temperature, the resultant product was extracted with EtOAc. The organic phase was washed with water, brine, dried (MgSO₄), filtered, and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a white solid (3.49 g, 99%); mp 63–64°C. ¹H NMR (500 MHz, CDCl₃) δ 10.07 (s, 1H), 8.56 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.98 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.51 (t, *J* = 7.6 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 1H), 4.27 (t, *J* = 7.3 Hz, 2H), 1.86 (p, *J* = 7.2 Hz, 2H), 1.34 (d, *J* = 3.7 Hz, 4H), 0.87 (t, *J* = 6.9 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 191.86 (C=O), 144.21 (C), 141.33 (C), 128.66 (C), 127.27 (CH), 126.85 (CH), 124.09 (CH), 123.21 (C), 123.15 (C), 120.87 (CH), 120.43 (CH), 109.55 (CH), 109.07 (CH), 43.54 (CH₂), 29.50 (CH₂), 28.78 (CH₂), 22.59 (CH₂), 14.08 (CH₃).

4.1.3. 9-Pentyl-9H-carbazole-3-carboxylic acid (3)—To an ice-cold solution of 9-pentyl-3-formylcarbazole (**2**) (2.96 g, 11.16 mmol) in water/acetone (100 mL, 1:1, v/v) was added dropwise under stirring a solution of potassium permanganate (1.8 g, 11.39 mmol) in acetone (50 mL). The mixture was heated 3 h at reflux and then allowed to cool to room temperature. After that the reaction mixture was quenched with ethanol (20 mL), and then stirred for 30 min at reflux. After cooling to room temperature, the mixture was filtered through a pad of Celite[®] and concentrated *in vacuo*. The concentrated solution was diluted with water (100 mL), basified with NaOH to pH *ca.* 10, and extracted with heptane/ether (4:1, v/v, 50 mL × 3) to remove the unreacted starting material. The aqueous solution was cooled on an ice-water bath and then acidified with ice-cold solution of sulfuric acid (20%) to pH *ca.* 2. The resultant bulky precipitate was extracted with EtOAc (150 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The precipitated product was collected by filtration, washed with heptanes (20 mL), and dried overnight to produce the title compound **3** (1.743 g, 55%) as a light yellow-to-greenish solid; mp 147°C. ¹H NMR (500 MHz, CDCl₃) δ 12.66 (s, 1H), 8.91 (d, *J* = 1.5 Hz, 1H), 8.25 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 7.54 – 7.47 (m, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 8.7 Hz, 1H), 7.34 – 7.26 (m, 1H), 4.27 (t, *J* = 7.3 Hz, 2H), 1.86 (p, *J* = 7.4 Hz, 2H), 1.40 – 1.29 (m, 4H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C NMR

and DEPT (126 MHz, CDCl₃) δ 173.58 (C=O), 143.80 (C), 141.22 (C), 128.04 (CH), 126.60 (CH), 123.91 (CH), 123.15 (C), 122.85 (C), 120.92 (CH), 120.17 (CH), 119.84 (C), 109.35 (CH), 108.43 (CH), 43.48 (CH₂), 29.55 (CH₂), 28.82 (CH₂), 22.65 (CH₂), 14.15 (CH₃).

4.1.4. (9-Pentyl-9H-carbazol-3-yl)(piperidin-1-yl)methanone (4)

Method A: amide coupling: Carboxylic acid **3** (300 mg, 1.07 mmol), piperidine (215 mg, 2.53 mmol), DIPEA (363 μL, 2.14 mmol), and DMAP (156 mg, 1.28 mmol) were added to DCM (30 mL) under nitrogen. The obtained solution was cooled down on an ice-water bath. EDC (350 mg, 1.83 mmol) was added to the solution, and the reaction mixture was stirred for 16 h while warming at room temperature. The solvent was removed *in vacuo*, and the obtained residue was extracted with EtOAc (100 mL). The organic layer was washed consecutively with 5% citric acid solution (50 mL × 3), concentrated sodium bicarbonate (50 mL × 3), brine (50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a light yellow gum (345 mg, 93%). ¹H NMR (500 MHz, CDCl₃) δ 8.19 (d, *J* = 1.2 Hz, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.49 – 7.43 (m, 1H), 7.41 – 7.35 (m, 2H), 7.26 – 7.20 (m, 1H), 4.25 (t, *J* = 7.2 Hz, 2H), 3.73 (br.m, 4H), 1.83 (p, *J* = 7.3 Hz, 2H), 1.72 – 1.65 (m, 3H), 1.61 (br.s, 3H), 1.36 – 1.29 (m, 4H), 0.85 (t, *J* = 7.0 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.53 (C=O), 140.99 (C), 140.90 (C), 126.74 (C), 126.13 (CH), 125.08 (CH), 122.74 (C), 122.43 (C), 120.54 (CH), 119.88 (CH), 119.28 (CH), 109.01 (CH), 108.40 (CH), 43.20 (CH₂), 29.41 (CH₂), 28.71 (CH₂), 24.79 (CH₂), 22.53 (CH₂), 14.03 (CH₃). ESI: *m/z* 349.5 (M + H)⁺. HRMS calcd for C₂₃H₂₉N₂O (M + H)⁺ 349.2280, found 349.2311.

4.1.5. N,N-diethyl-9-pentyl-9H-carbazole-3-carboxamide (5)—Using carboxylic acid **3** (112 mg, 0.40 mmol) and diethylamine (74 μL, 0.71 mmol) as starting compounds, the title compound was prepared as a clear, colorless viscous oil according to the procedure described above for **4**. Yield: 44 mg (33%). ¹H NMR (500 MHz, CDCl₃) δ 8.16 (d, *J* = 1.5 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.52 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.26 – 7.22 (m, 1H), 4.30 (t, *J* = 7.2 Hz, 2H), 3.83 – 3.16 (br. m, 4H), 1.87 (p, *J* = 7.4 Hz, 2H), 1.35 (dq, *J* = 7.2, 3.6 Hz, 4H), 1.31 – 1.07 (m, 6H), 0.87 (t, *J* = 7.1 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 172.59 (C=O), 141.00 (C), 140.94 (C), 127.71 (C), 126.23 (CH), 124.61 (CH), 122.87 (C), 122.53 (C), 120.67 (CH), 119.36 (CH), 119.28 (CH), 109.11 (CH), 108.58 (CH), 43.36 (CH₂), 29.57 (CH₂), 28.85 (CH₂), 22.67 (CH₂), 14.16 (CH₃). ESI: *m/z* 337.2 (M + H)⁺. HRMS calcd for C₂₂H₂₉N₂O (M + H)⁺ 337.2280, found 337.2309.

4.1.6. (9-Pentyl-9H-carbazol-3-yl)(1,1-dioxo-thiomorpholino)methanone (6)—Using carboxylic acid **3** (115 mg, 0.41 mmol) and 1,1-dioxo-thiomorpholine (80 mg, 0.59 mmol) as starting compounds, the title compound was prepared as a clear, colorless gum according to the procedure described above for **4**. The obtained product solidified upon refrigeration to a white solid Yield: 83 mg (51%); mp 115°C. ¹H NMR (500 MHz, CDCl₃) δ 8.23 (d, *J* = 1.5 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.55 (dd, *J* = 8.4, 1.7 Hz, 1H), 7.51 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H), 7.43 (t, *J* = 8.3 Hz, 2H), 7.30 – 7.24 (m, 1H), 4.30 (t, *J* = 7.3 Hz, 2H), 4.17 (s, 4H), 3.10 (s, 4H), 1.86 (p, *J* = 7.4 Hz, 2H), 1.35 (dq, *J* = 7.2, 3.7 Hz, 4H), 0.91 – 0.83 (m, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 172.50 (C=O), 141.69 (C), 141.08 (C), 126.72 (CH), 125.12 (CH), 123.87 (C), 122.79 (C), 122.49 (C), 120.72 (CH), 120.47 (CH), 119.83 (CH), 109.31 (CH), 108.91 (CH), 52.18 (CH₂), 43.38 (CH₂), 29.46 (CH₂), 28.76 (CH₂), 22.57 (CH₂), 14.09 (CH₃). ESI: *m/z* 399.1 (M + H)⁺. HRMS calcd for C₂₂H₂₇N₂O₃S (M + H)⁺ 399.1742, found 399.1718.

4.1.7. 9-Pentyl-N-(piperidin-1-yl)-9H-carbazole-3-carboxamide (7)—Using carboxylic acid **3** (100 mg, 0.36 mmol) and 1-aminopiperidine (39 μ L, 0.36 mmol) as starting compounds, the title compound was prepared as a light yellow solid according to the procedure described above for **4** with the exception of not using 5% citric acid wash during the workup. Yield: 109 mg (84%); mp 158°C. ^1H NMR (500 MHz, CDCl_3) δ 8.51 (s, 1H), 8.11 (d, $J = 7.7$ Hz, 1H), 7.86 (d, $J = 8.3$ Hz, 1H), 7.49 (ddd, $J = 8.2, 7.1, 1.0$ Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 1H), 7.36 (d, $J = 8.6$ Hz, 1H), 7.26 (dd, $J = 8.1, 6.8$ Hz, 1H), 6.96 (s, 1H), 4.28 (t, $J = 7.2$ Hz, 2H), 2.93 (br. s, 4H), 1.85 (p, $J = 7.5$ Hz, 2H), 1.82 – 1.73 (m, 4H), 1.46 (br. s, 2H), 1.38 – 1.30 (m, 4H), 0.86 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 166.22 (C=O), 142.29 (C), 141.06 (C), 126.35 (CH), 124.90 (CH), 124.62 (C), 122.92 (C), 122.64 (C), 120.75 (CH), 120.02 (CH), 119.63 (CH), 109.16 (CH), 108.49 (CH), 57.45 (CH_2), 43.31 (CH_2), 29.47 (CH_2), 28.75 (CH_2), 25.57 (CH_2), 23.50 (CH_2), 22.58 (CH_2), 14.09 (CH_3). ESI: m/z 364.2 (M + H) $^+$. HRMS calcd for $\text{C}_{23}\text{H}_{30}\text{N}_3\text{O}$ (M + H) $^+$ 364.2389, found 364.2356.

4.1.8. Morpholino(9-pentyl-9H-carbazol-3-yl)methanone (8)—Using carboxylic acid **3** (100 mg, 0.36 mmol) and morpholine (62 μ L, 0.71 mmol) as starting compounds, the title compound was prepared as a white solid according to the procedure described above for **4**. Yield: 109 mg (84%); mp 98°C. ^1H NMR (500 MHz, CDCl_3) δ 8.21 (d, $J = 1.6$ Hz, 1H), 8.09 (d, $J = 7.7$ Hz, 1H), 7.54 (dd, $J = 8.4, 1.7$ Hz, 1H), 7.48 (ddd, $J = 8.2, 7.1, 1.2$ Hz, 1H), 7.40 (t, $J = 8.5$ Hz, 2H), 7.25 (ddd, $J = 7.9, 7.0, 0.9$ Hz, 1H), 4.28 (t, $J = 7.2$ Hz, 2H), 3.73 (s, 8H), 1.90 – 1.79 (m, 2H), 1.38 – 1.28 (m, 4H), 0.86 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 171.72 (C=O), 141.26 (C), 140.98 (C), 126.36 (CH), 125.45 (C), 125.29 (CH), 122.66 (C), 122.57 (C), 120.63 (CH), 120.29 (CH), 119.50 (CH), 109.14 (CH), 108.59 (CH), 67.11 (CH_2), 43.28 (CH_2), 29.45 (CH_2), 28.74 (CH_2), 22.57 (CH_2), 14.08 (CH_3). ESI: m/z 351.2 (M + H) $^+$. HRMS calcd for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_2$ (M + H) $^+$ 351.2073, found 351.2071.

4.1.9. (4-Methylpiperazin-1-yl)(9-pentyl-9H-carbazol-3-yl)methanone (9)—Using carboxylic acid **3** (110 mg, 0.39 mmol) and 1-methylpiperazine (71 mg, 0.71 mmol) as starting compounds, the title compound was prepared as a clear, orange viscous oil according to the procedure described above for **4** with the exception of not using 5% citric acid during the workup. Yield: 134 mg (94%). ^1H NMR (500 MHz, CDCl_3) δ 8.20 (d, $J = 1.3$ Hz, 1H), 8.09 (d, $J = 7.7$ Hz, 1H), 7.55 (dd, $J = 8.4, 1.6$ Hz, 1H), 7.48 (ddd, $J = 8.2, 7.1, 1.1$ Hz, 1H), 7.41 (d, $J = 8.3$ Hz, 1H), 7.39 (d, $J = 8.5$ Hz, 1H), 7.27 – 7.22 (m, 1H), 4.28 (t, $J = 7.2$ Hz, 2H), 3.78 (br. s, 4H), 2.46 (br. s, 4H), 2.33 (s, 3H), 1.85 (p, $J = 7.3$ Hz, 2H), 1.37 – 1.32 (m, 4H), 0.86 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 171.63 (C=O), 141.19 (C), 140.97 (C), 126.30 (CH), 125.97 (C), 125.31 (CH), 122.72 (C), 122.51 (C), 120.64 (CH), 120.21 (CH), 119.44 (CH), 109.11 (CH), 108.52 (CH), 46.21 (CH_3), 43.29 (CH_2), 29.47 (CH_2), 28.76 (CH_2), 22.58 (CH_2), 14.09 (CH_3). ESI: m/z 364.2 (M + H) $^+$. HRMS calcd for $\text{C}_{23}\text{H}_{30}\text{N}_3\text{O}$ (M + H) $^+$ 364.2389, found 364.2356.

4.1.10. N-(1-adamantyl)-9-pentyl-9H-carbazole-3-carboxamide (10)—Using carboxylic acid **3** (112 mg, 0.40 mmol) and 1-adamantylamine (74 μ L, 0.71 mmol) as starting compounds, the title compound was prepared as a light yellow glass according to the procedure described above for compound **4**. Yield: 91 mg (62%). ^1H NMR (500 MHz, CDCl_3) δ 8.48 (d, $J = 1.7$ Hz, 1H), 8.11 (d, $J = 7.7$ Hz, 1H), 7.85 (dd, $J = 8.6, 1.8$ Hz, 1H), 7.48 (ddd, $J = 8.2, 7.1, 1.2$ Hz, 1H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.35 (d, $J = 8.6$ Hz, 1H), 7.29 – 7.22 (m, 1H), 5.97 (s, 1H), 4.26 (t, $J = 7.2$ Hz, 2H), 2.20 (d, $J = 2.6$ Hz, 6H), 2.15 (br. s, 3H), 1.88 – 1.80 (m, 2H), 1.79 – 1.69 (m, 6H), 1.37 – 1.28 (m, 4H), 0.85 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 167.51 (C=O), 142.14 (C), 141.13 (C), 126.79 (C), 126.31 (CH), 124.69 (CH), 123.03 (C), 122.62 (C), 120.70 (CH), 119.59 (CH),

119.56 (CH), 109.19 (CH), 108.41 (CH), 52.36 (C), 43.36 (CH₂), 42.01 (CH₂), 36.64 (CH₂), 29.74 (CH), 29.51 (CH₂), 28.80 (CH₂), 22.62 (CH₂), 14.13 (CH₃). ESI: m/z 415.2 (M + H)⁺. HRMS calcd for C₂₈H₃₅N₂O (M + H)⁺ 415.2749, found 415.2762.

4.1.11. *N*-(4-chlorophenethyl)-9-pentyl-9*H*-carbazole-3-carboxamide (11)—

Using carboxylic acid **3** (105 mg, 0.37 mmol) and 2-(4-chlorophenyl)ethanamine (90 mg, 0.58 mmol) as starting compounds, the title compound was prepared as a white solid according to the procedure described above for **4**. Yield: 38 mg (23%); mp 153°C. ¹H NMR (500 MHz, CDCl₃) δ 8.57 – 8.43 (m, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.81 (ddd, J = 8.6, 1.7, 0.8 Hz, 1H), 7.49 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.30 – 7.22 (m, 3H), 7.17 (d, J = 8.4 Hz, 2H), 4.26 (t, J = 7.3 Hz, 2H), 3.76 – 3.70 (m, 2H), 2.94 (t, J = 7.0 Hz, 2H), 1.84 (p, J = 7.4 Hz, 2H), 1.37 – 1.31 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 168.39 (C=O), 168.32 (C=O), 142.37 (C), 141.17 (C), 137.85 (C), 132.46 (C), 130.42 (CH), 128.92 (CH), 126.49 (CH), 125.15 (C), 125.12 (C), 124.65 (CH), 122.96 (C), 122.77 (C), 120.74 (CH), 119.90 (CH), 119.76 (CH), 109.25 (CH), 108.58 (CH), 43.42 (CH₂), 41.38 (CH₂), 41.26 (CH₂), 35.47 (CH₂), 35.46 (CH₂), 29.54 (CH₂), 28.82 (CH₂), 22.63 (CH₂), 14.14 (CH₃). ESI: m/z 419.4 (M + H)⁺. HRMS calcd for C₂₆H₂₈N₂OCl (M + H)⁺ 419.1890, found 419.1870.

4.1.12. *Tert*-butyl 4-((9-pentyl-9*H*-carbazole-3-carboxamido)methyl)piperidine-1-carboxylate (12)—

Carboxylic acid **3** (700 mg, 2.49 mmol), HOBt (404 mg, 2.99 mmol), DIPEA (847 μL, 4.98 mmol), DMAP (365 mg, 2.99 mmol), and EDC (573 mg, 2.99 mmol) were added upon stirring to DCM (50 mL) under nitrogen. The obtained solution was cooled down on an ice-water bath. *Tert*-butyl 4-(aminomethyl)piperidine-1-carboxylate (749 mg, 2.99 mmol) was added in one portion, and the resulting reaction mixture was then allowed to warm to room temperature and stirred for 16 h. The solvent was removed *in vacuo*, and the obtained residue was extracted with EtOAc (100 mL). The organic layer was washed consecutively with 5% citric acid solution (50 mL × 3), concentrated sodium bicarbonate (50 mL × 3), and brine (50 mL). The organic layer was then separated, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography eluting with heptane/EtOAc (gradient elution) to give 1.096 g (92%) of **12** as a yellow glass. ¹H NMR (400 MHz, CDCl₃) δ 8.56 (d, J = 1.1 Hz, 1H), 8.08 (d, J = 7.7 Hz, 1H), 7.90 (dd, J = 8.6, 1.4 Hz, 1H), 7.47 (ddd, J = 8.3, 7.1, 1.2 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.24 (t, J = 7.5 Hz, 1H), 6.64 (br s, 1H), 4.26 (t, J = 7.2 Hz, 2H), 4.07 (br m, 2H), 3.39 (br s, 2H), 2.68 (t, J = 12.1 Hz, 2H), 1.91 – 1.79 (m, 3H), 1.75 (br d, J = 13.2 Hz, 2H), 1.45 (s, 9H), 1.37 – 1.29 (m, J = 8.9, 5.2 Hz, 4H), 1.20 (qd, J = 12.5, 4.3 Hz, 2H), 0.86 (t, J = 6.8 Hz, 3H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 168.49 (C=O), 154.76 (C), 142.05 (C), 140.86 (C), 126.12 (CH), 125.05 (C), 124.86 (CH), 122.76 (C), 122.43 (C), 120.43 (CH), 119.80 (CH), 119.38 (CH), 108.96 (CH), 108.19 (CH), 79.24 (C), 45.45 (CH₂), 43.00 (CH₂), 36.48 (CH), 29.91 (CH₂), 29.20 (CH₂), 28.50 (CH₂), 28.41 (CH₃), 22.33 (CH₂), 13.87 (CH₃). ESI: m/z 478.2 (M + H)⁺. HRMS calcd for C₂₉H₄₀N₃O₃ (M + H)⁺ 478.3070, found 478.3007.

4.1.13. *Tert*-butyl 4-(9-pentyl-9*H*-carbazole-3-carbonyl)piperazine-1-carboxylate (13)—

Using carboxylic acid **3** (700 mg, 2.49 mmol) and *tert*-butyl piperazine-1-carboxylate (556 mg, 2.99 mmol) as starting compounds, the title compound was prepared as a light yellow glass according to the procedure described above for compound **14**. Yield: 1.03 g (92%). ¹H NMR (400 MHz, CDCl₃) δ 8.20 (d, J = 1.2 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.54 (dd, J = 8.4, 1.5 Hz, 1H), 7.48 (J = 8.3, 7.0, 1.2 Hz, ddd, 1H), 7.41 (t, J = 8.1 Hz, 2H), 7.26 (ddd, J = 7.0, 5.9, 1.1 Hz, 1H), 4.30 (t, J = 7.2 Hz, 2H), 3.68 (br s, 4H), 3.50 (br s, 4H), 1.93 – 1.82 (m, 2H), 1.48 (s, 9H), 1.39 – 1.31 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 171.98 (C=O), 154.83 (C), 141.38

(C), 141.09 (C), 126.45 (CH), 125.71 (C), 125.32 (CH), 122.77 (C), 122.69 (C), 120.70 (CH), 120.35 (CH), 119.59 (CH), 109.20 (CH), 108.66 (CH), 80.42 (C), 43.39 (CH₂), 29.53 (CH₂), 28.81 (CH₂), 28.56 (CH₃), 22.62 (CH₂), 14.11 (CH₃). ESI: m/z 450.1 (M + H)⁺. HRMS calcd for C₂₇H₃₆N₃O₃ (M + H)⁺ 450.2757, found 450.2777.

4.1.14. 9-Pentyl-N-(piperidin-4-ylmethyl)-9H-carbazole-3-carboxamide (14)—

Dry hydrogen chloride gas was passed through a solution containing 760 mg (1.59 mmol) of **12** dissolved in 30 mL of EtOAc for about ten minutes. The solution was stirred overnight, and then concentrated *in vacuo*. The residue was dissolved in EtOAc (100 mL) and washed with 1N NaOH. The organic layer was washed with brine (30 mL), dried (MgSO₄), and filtered. The solvent was evaporated under reduced pressure, and the residue self-crystallized. The white crystalline solid was collected by filtration, washed with heptanes (20 mL) and dried *in vacuo*. Yield (as a free base): 527 mg (88%); mp 163°C. ¹H NMR (400 MHz, CDCl₃) δ 8.55 (d, J = 1.4 Hz, 1H), 8.09 (d, J = 7.7 Hz, 1H), 7.90 (dd, J = 8.6, 1.7 Hz, 1H), 7.48 (ddd, J = 8.3, 7.1, 1.2 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.24 (ddd, J = 8.0, 7.0, 1.0 Hz, 1H), 6.57 (t, J = 5.7 Hz, 1H), 4.26 (t, J = 7.2 Hz, 2H), 3.39 (t, J = 6.2 Hz, 2H), 3.09 (d, J = 12.1 Hz, 2H), 2.60 (td, J = 12.2, 2.5 Hz, 2H), 1.98 (s, 1H), 1.91 – 1.80 (m, 2H), 1.80 – 1.71 (m, 3H), 1.40 – 1.29 (m, 4H), 1.23 (qd, J = 12.8, 3.7 Hz, 2H), 0.86 (t, J = 6.9 Hz, 3H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 168.51 (C=O), 142.32 (C), 141.15 (C), 126.40 (CH), 125.41 (C), 124.79 (CH), 122.99 (C), 122.73 (C), 120.73 (CH), 119.82 (CH), 119.67 (CH), 109.21 (CH), 108.50 (CH), 46.47 (CH₂), 46.20 (CH₂), 43.37 (CH₂), 36.91 (CH), 31.37 (CH₂), 29.49 (CH₂), 28.77 (CH₂), 22.59 (CH₂), 14.09 (CH₃). ESI: m/z 378.2 (M + H)⁺. HRMS calcd for C₂₄H₃₂N₃O (M + H)⁺ 378.2545, found 378.2583.

4.1.15. (9-Pentyl-9H-carbazol-3-yl)(piperazin-1-yl)methanone (15)—Starting with compound **13** (973 mg, 2.16 mmol), the title compound was prepared as a light yellow glass according to the procedure described above for compound **14**. Yield (as a free base): 732 mg (97%). ¹H NMR (400 MHz, CDCl₃) δ 8.23 (d, J = 1.1 Hz, 1H), 8.13 (d, J = 7.7 Hz, 1H), 7.57 (dd, J = 8.4, 1.5 Hz, 1H), 7.52 (ddd, J = 8.3, 7.0, 1.2 Hz, 1H), 7.44 (t, J = 8.6 Hz, 2H), 7.29 (ddd, J = 7.9, 7.0, 1.0 Hz, 1H), 4.33 (t, J = 7.2 Hz, 2H), 3.72 (br s, 4H), 2.95 (br s, 4H), 2.02 (s, 1H), 1.90 (p, J = 7.3 Hz, 2H), 1.45 – 1.31 (m, 4H), 0.90 (t, J = 7.0 Hz, 3H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 171.74 (C=O), 141.20 (C), 141.04 (C), 126.32 (CH), 126.18 (C), 125.31 (CH), 122.80 (C), 122.60 (C), 120.67 (CH), 120.21 (CH), 119.47 (CH), 109.13 (CH), 108.55 (CH), 46.47 (CH₂), 43.34 (CH₂), 29.50 (CH₂), 28.78 (CH₂), 22.60 (CH₂), 14.09 (CH₃). ESI: m/z 350.1 (M + H)⁺. HRMS calcd for C₂₂H₂₈N₃O (M + H)⁺ 350.2232, found 350.2235.

4.1.16. Pyridin-4-yl-methyl 9-pentyl-9H-carbazole-3-carboxylate (16)—To a stirred mixture of carboxylic acid **3** (200 mg, 0.71 mmol), 4-(bromomethyl)pyridine (147 mg, 0.85 mmol), triethylamine (178 μL, 1.27 mmol), and sodium carbonate (1 g, 9.43 mmol) in THF (3 mL) in a 20 mL microwave vessel was added 1 M TBAF in THF (853 μL, 0.853 mmol). The mixture was stirred at room temperature for 16 h under a nitrogen atmosphere, and then subjected to microwave irradiation at 60°C for 1 h. After cooling to room temperature, the reaction mixture was diluted with DCM (30 mL) and transferred to a round-bottomed flask. The volatiles were removed *in vacuo*, and the obtained residue was dissolved in DCM (150 mL). The organic layer was washed with 0.05 M NaOH (50 mL × 2), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The obtained residue was purified by flash chromatography eluting with heptane/EtOAc (gradient elution) on a Biotage® KP-NH cartridge yielding compound **12** as a yellowish oil that solidified on standing to a white solid. Yield: 203 mg, (77%); mp 104°C. ¹H NMR (400 MHz, CDCl₃) δ 8.87 (s, 1H), 8.68 (br. s, 2H), 8.22 (d, J = 8.6 Hz, 1H), 8.16 (d, J = 7.7 Hz, 1H), 7.58 – 7.48

(m, 3H), 7.44 (d, $J = 7.5$ Hz, 1H), 7.42 (d, $J = 8.4$ Hz, 1H), 7.30 (t, $J = 7.4$ Hz, 1H), 5.48 (s, 2H), 4.32 (t, $J = 7.2$ Hz, 2H), 1.98 – 1.80 (m, 2H), 1.45 – 1.29 (m, 4H), 0.88 (t, $J = 6.7$ Hz, 3H). ^{13}C and APT NMR (101 MHz, CDCl_3) δ 166.97 (C=O), 148.37 (CH), 148.27 (C), 143.63 (CH), 141.28 (C), 127.56 (CH), 126.75 (CH), 123.34 (CH), 123.06 (C), 122.95 (C), 122.61 (CH), 120.88 (CH), 120.22 (CH), 119.73 (C), 109.43 (CH), 108.60 (CH), 64.37 (CH_2), 43.53 (CH_2), 29.53 (CH_2), 28.80 (CH_2), 22.61 (CH_2), 14.10 (CH_3). ESI: m/z 373.2 ($\text{M} + \text{H}$)⁺. HRMS calcd for $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_2$ ($\text{M} + \text{H}$)⁺ 373.1916, found 373.1890.

4.1.17. Pyridin-2-ylmethyl 9-pentyl-9H-carbazole-3-carboxylate (17)—The title compound was prepared from carboxylic acid **3** (316 mg, 1.12 mmol) as a light amber viscous oil by the same procedure as described for **16**. Yield: 392 mg, (99%). ^1H NMR (500 MHz, CDCl_3) δ 8.86 (d, $J = 1.5$ Hz, 1H), 8.58 (ddd, $J = 4.8, 1.1, 0.7$ Hz, 1H), 8.20 (dd, $J = 8.6, 1.7$ Hz, 1H), 8.07 (d, $J = 7.7$ Hz, 1H), 7.62 (td, $J = 7.7, 1.8$ Hz, 1H), 7.46 (d, $J = 7.8$ Hz, 1H), 7.43 (ddd, $J = 8.2, 7.2, 1.1$ Hz, 1H), 7.31 (d, $J = 8.2$ Hz, 1H), 7.28 (d, $J = 8.7$ Hz, 1H), 7.22 (ddd, $J = 7.8, 7.1, 0.7$ Hz, 1H), 7.14 (ddd, $J = 7.5, 4.9, 0.9$ Hz, 1H), 5.55 (s, 2H), 4.11 (t, $J = 7.2$ Hz, 2H), 1.74 (p, $J = 7.3$ Hz, 2H), 1.33 – 1.17 (m, 4H), 0.80 (t, $J = 6.9$ Hz, 3H). ^{13}C and APT NMR (126 MHz, CDCl_3) δ 166.77 (C=O), 156.23 (C), 149.00 (CH), 143.01 (C), 140.79 (C), 136.80 (CH), 127.24 (CH), 126.24 (CH), 122.90 (CH), 122.69 (C), 122.64 (CH), 122.40 (C), 121.59 (CH), 120.45 (CH), 119.91 (C), 119.71 (CH), 109.01 (CH), 108.09 (CH), 66.65 (CH_2), 42.93 (CH_2), 29.11 (CH_2), 28.41 (CH_2), 22.26 (CH_2), 13.81 (CH_3). ESI: m/z 373.1 ($\text{M} + \text{H}$)⁺. HRMS calcd for $\text{C}_{24}\text{H}_{25}\text{N}_2\text{O}_2$ ($\text{M} + \text{H}$)⁺ 373.1916, found 373.1890.

4.1.18. (9-Pentyl-9H-carbazol-3-yl)(phenyl)methanone (18)—Under argon atmosphere, AlCl_3 (309 mg, 2.32 mmol) was added to a solution of 9-pentyl-9H-carbazole (**1**) (500 mg, 2.11 mmol) in anhydrous benzene (30 mL), and the obtained solution was cooled by an ice bath for 20 min. Benzoyl chloride (282 μL , 2.43 mmol) was added dropwise *via* a syringe to the solution, and the reaction mixture was stirred for 16 h while warming at room temperature. The reaction mixture was cooled on an ice-water bath then poured onto a mixture of ice and 4 M NaOH solution (50 mL) and extracted with diethyl ether (150 mL). The organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO_4), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with EtOAc/heptanes in different proportions to give the target product (514 mg, 71%) as a light yellow solid: mp 116°C. ^1H NMR (500 MHz, CDCl_3) δ 8.61 (d, $J = 1.0$ Hz, 1H), 8.10 (d, $J = 7.7$ Hz, 1H), 8.03 (dd, $J = 8.5, 1.3$ Hz, 1H), 7.85 (d, $J = 7.1$ Hz, 2H), 7.59 (t, $J = 7.4$ Hz, 1H), 7.56 – 7.47 (m, 3H), 7.44 (d, $J = 2.2$ Hz, 1H), 7.43 (d, $J = 2.9$ Hz, 1H), 7.27 (t, $J = 7.4$ Hz, 1H), 4.32 (t, $J = 7.2$ Hz, 2H), 1.89 (p, $J = 7.0$ Hz, 2H), 1.46 – 1.29 (m, 4H), 0.88 (t, $J = 6.7$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 196.79 (C=O), 143.21 (C), 141.29 (C), 139.22 (C), 131.82 (CH), 130.08 (CH), 128.61 (CH), 128.59 (C), 128.35 (CH), 126.60 (CH), 124.22 (CH), 123.28 (C), 122.59 (C), 120.89 (CH), 120.08 (CH), 109.41 (CH), 108.45 (CH), 43.51 (CH_2), 29.55 (CH_2), 28.85 (CH_2), 22.65 (CH_2), 14.14 (CH_3). ESI: m/z 342.1 ($\text{M} + \text{H}$)⁺. HRMS calcd for $\text{C}_{24}\text{H}_{24}\text{NO}$ ($\text{M} + \text{H}$)⁺ 342.1858, found 342.1859.

4.1.19. 2-Bromo-1-(9-pentyl-9H-carbazol-3-yl)ethanone (19)—Under argon atmosphere, anhydrous AlCl_3 (658 mg, 4.93 mmol) was added to a solution of 9-pentyl-9H-carbazole (**1**) (1.172 g, 4.94 mmol) in anhydrous benzene (20 mL), and the obtained solution was cooled by an ice bath for 20 min. 1-Bromoacetyl bromide (429 μL , 4.94 mmol) was added dropwise to the solution, and the reaction mixture was stirred for 19 h while warming at room temperature. The reaction mixture was quenched with 3 mL of concentrated HCl solution, and then extracted with diethyl ether (150 mL). The organic phase was washed with saturated solution of ascorbic acid (3 \times 30 mL), water (30 mL), saturated aqueous

sodium bicarbonate (2 × 30 mL), brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The residue was purified on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a light yellow gum (522 mg, 67%) which self-crystallized shortly after standing: mp 91°C. ¹H NMR (500 MHz, CDCl₃) δ 8.75 (d, *J* = 1.7 Hz, 1H), 8.15 (d, *J* = 7.8 Hz, 1H), 8.12 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.53 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 7.44 (d, *J* = 8.2 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 1H), 7.32 (ddd, *J* = 7.8, 7.0, 0.7 Hz, 1H), 4.58 (s, 2H), 4.30 (t, *J* = 7.3 Hz, 2H), 1.88 (p, *J* = 7.4 Hz, 2H), 1.36 (dq, *J* = 7.2, 3.6 Hz, 4H), 0.88 (t, *J* = 7.1 Hz, 1H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 190.94 (C=O), 143.74 (C), 141.33 (C), 127.11 (CH), 126.89 (CH), 125.32 (C), 123.17 (C), 122.99 (C), 122.75 (CH), 120.89 (CH), 120.43 (CH), 109.55 (CH), 108.80 (CH), 43.54 (CH₂), 31.43 (CH₂), 29.52 (CH₂), 28.82 (CH₂), 22.62 (CH₂), 14.13 (CH₃).

4.1.20. 1-(9-Pentyl-9H-carbazol-3-yl)-2-(piperidin-1-yl)ethanone (20)—Under nitrogen atmosphere, a mixture of bromide **19** (208 mg, 0.58 mmol), piperidine (165 μL, 1.67 mmol), and triethylamine (234 μL, 1.68 mmol) in DMF (3 mL) was subjected to microwave irradiation at 90°C for 5 min. The mixture was allowed to cool to room temperature, and the organic solvents were evaporated *in vacuo*. The residue was purified on a Biotage® KP-NH cartridge (amino-modified silica gel) using cyclohexane/EtOAc in different proportions to afford to give the title compound as a clear yellowish oil (202 mg, 100%), which darkened on standing. ¹H NMR (500 MHz, CDCl₃) δ 8.80 (d, *J* = 0.8 Hz, 1H), 8.15 (dd, *J* = 8.7, 1.0 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.45 (dd, *J* = 8.0, 7.3 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 7.25 (t, *J* = 7.5 Hz, 1H), 4.16 (t, *J* = 7.2 Hz, 2H), 3.88 (s, 2H), 2.59 (br. s, 4H), 1.84 – 1.73 (m, 2H), 1.72 – 1.61 (m, 4H), 1.51 – 1.40 (m, 2H), 1.34 – 1.23 (m, 4H), 0.83 (t, *J* = 6.8 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 196.04 (C=O), 143.06 (C), 140.97 (C), 127.63 (C), 126.30 (CH), 126.23 (CH), 123.10 (C), 122.38 (C), 121.55 (CH), 120.49 (CH), 119.82 (CH), 109.15 (CH), 108.12 (CH), 65.40 (CH₂), 54.95 (CH₂), 43.09 (CH₂), 29.25 (CH₂), 28.56 (CH₂), 25.87 (CH₂), 24.08 (CH₂), 22.39 (CH₂), 13.91 (CH₃). ESI: *m/z* 363.1 (M + H)⁺. HRMS calcd for C₂₄H₃₁N₂O (M + H)⁺ 363.2436, found 363.2408.

4.1.21. 1-(9-Pentyl-9H-carbazol-3-yl)-2-(1,1-dioxo-thiomorpholino)ethanone (21)—Using bromide **19** (200 mg, 0.56 mmol), 1,1-dioxo-thiomorpholine (226 mg, 1.67 mmol), and triethylamine (234 μL, 1.68 mmol) as starting compounds, the title compound was prepared as a beige glass according to the procedure described above for **20**. Yield: 207 mg (90%). ¹H NMR (500 MHz, CDCl₃) δ 8.68 (d, *J* = 1.5 Hz, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 8.05 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.52 – 7.47 (m, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.35 (d, *J* = 8.7 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 4.12 (s, 2H), 3.25 – 3.17 (m, 4H), 3.17 – 3.09 (m, 4H), 1.89 – 1.75 (m, 2H), 1.38 – 1.26 (m, 4H), 0.85 (t, *J* = 6.9 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 194.76 (C=O), 143.24 (C), 140.98 (C), 126.63 (C), 126.58 (CH), 125.80 (CH), 122.83 (C), 122.45 (C), 121.25 (CH), 120.51 (CH), 120.04 (CH), 109.33 (CH), 108.46 (CH), 62.20 (CH₂), 51.46 (CH₂), 51.10 (CH₂), 43.14 (CH₂), 29.18 (CH₂), 28.50 (CH₂), 22.32 (CH₂), 13.90 (CH₃). ESI: *m/z* 413.1 (M + H)⁺. HRMS calcd for C₂₃H₂₉N₂O₃S (M + H)⁺ 413.1899, found 413.1874.

4.1.22. 2-(4-Methylpiperazin-1-yl)-1-(9-pentyl-9H-carbazol-3-yl)ethanone (22)—Using bromide **19** (265 mg, 0.74 mmol), 1-methylpiperazine (214 μL, 1.93 mmol), and triethylamine (269 μL, 1.93 mmol) as starting compounds, the title compound was prepared as a clear amber gum according to the procedure described above for **20**. Yield: 247 mg (89%). ¹H NMR (500 MHz, CDCl₃) δ 8.73 (d, *J* = 1.5 Hz, 1H), 8.11 (d, *J* = 7.4 Hz, 1H), 8.09 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.47 (ddd, *J* = 8.1, 6.9, 0.9 Hz, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.26 (t, *J* = 7.4 Hz, 1H), 4.18 (t, *J* = 7.2 Hz, 2H), 3.98 (s, 2H), 3.08 – 2.62 (br. m, 8H), 2.48 (s, 3H), 1.84 – 1.74 (m, 2H), 1.35 – 1.23 (m, 4H), 0.84 (t, *J* = 6.9

Hz, 3H). ^{13}C and DEPT NMR (101 MHz, CDCl_3) δ 194.89 (C=O), 142.97 (C), 140.79 (C), 126.99 (C), 126.26 (CH), 125.81 (CH), 122.79 (C), 122.24 (C), 121.21 (CH), 120.34 (CH), 119.76 (CH), 109.04 (CH), 108.11 (CH), 63.60 (CH_2), 54.26 (CH_2), 52.11 (CH_2), 44.88 (CH_3), 42.93 (CH_2), 29.02 (CH_2), 28.33 (CH_2), 22.15 (CH_2), 13.71 (CH_3). ESI: m/z 378.2 (M + H) $^+$. HRMS calcd for $\text{C}_{24}\text{H}_{32}\text{N}_3\text{O}$ (M + H) $^+$ 378.2545, found 378.2565.

4.1.23. 1,1-Dimethyl-4-[(9-pentyl-9H-carbazol-3-yl)carbonyl]piperazin-1-ium iodide (23)—Methyl iodide (764 μL , 12.32 mmol) was added to a stirred solution of the tertiary amine **9** (320 mg, 0.88 mmol) in anhydrous diethyl ether (10 mL). A precipitate immediately started forming, and stirring was continued for 18 h at room temperature. The precipitated solid was isolated by filtration, washed with diethyl ether (*ca.* 50 mL), and dried under high vacuum to provide the title compound (186 mg, 42%) as a light yellow solid: mp 119°C (with decomposition). ^1H NMR (500 MHz, CDCl_3) δ 8.37 (s, 1H), 8.14 (d, $J = 7.7$ Hz, 1H), 7.55 (d, $J = 8.6$ Hz, 1H), 7.40 (t, $J = 7.7$ Hz, 1H), 7.32 (d, $J = 8.3$ Hz, 1H), 7.27 (d, $J = 9.4$ Hz, 1H), 7.15 (t, $J = 7.4$ Hz, 1H), 4.13 (t, $J = 6.7$ Hz, 2H), 3.97 (s, 4H), 3.78 (s, 4H), 3.49 (s, 6H), 1.73 (dt, $J = 14.6, 7.4$ Hz, 2H), 1.30 – 1.21 (m, 4H), 0.81 (t, $J = 6.8$ Hz, 3H). ^{13}C and DEPT NMR (126 MHz, CDCl_3) δ 171.73 (C=O), 141.39 (C), 140.91 (C), 126.56 (CH), 125.51 (CH), 123.77 (C), 122.54 (C), 122.49 (C), 121.25 (CH), 120.92 (CH), 119.71 (CH), 109.15 (CH), 109.04 (CH), 61.31 (CH_2), 52.26 (CH_3), 43.28 (CH_2), 29.35 (CH_2), 28.67 (CH_2), 22.50 (CH_2), 14.09 (CH_3). ESI: m/z 378.1 (M + H) $^+$. HRMS calcd for $\text{C}_{24}\text{H}_{32}\text{N}_3\text{O}$ (M + H) $^+$ 378.2545, found 378.2523.

4.1.24. 9-Pentyl-9H-carbazole-3-carbonitrile (24)—A mixture of 9-pentyl-9H-carbazole-3-carbaldehyde (500 mg, 1.88 mmol), hydroxylamine hydrochloride (270 mg, 4.19 mmol), and *p*-toluenesulfonic acid (73 mg, 0.42 mmol) was stirred at 235°C in a 20 mL microwave vessel under a stream of nitrogen until foaming and evolution of gas ceased. The mixture was then subjected to microwave irradiation at 105°C for 5 min. The reaction mixture was allowed to cool to room temperature, and then heated again at 235°C under a stream of nitrogen for 20 min. The obtained residue was purified by column chromatography on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a light orange solid. Yield: 336 mg (68%), mp 71°C. ^1H NMR (400 MHz, CDCl_3) δ 8.26 (d, $J = 1.3$ Hz, 1H), 8.01 (d, $J = 7.8$ Hz, 1H), 7.62 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.51 (ddd, $J = 8.4, 7.1, 1.3$ Hz, 1H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.34 (d, $J = 8.5$ Hz, 1H), 7.27 (t, $J = 7.5$ Hz, 1H), 4.21 (t, $J = 7.3$ Hz, 2H), 1.81 (p, $J = 7.3$ Hz, 2H), 1.42 – 1.18 (m, 4H), 0.85 (t, $J = 6.9$ Hz, 3H). ^{13}C and APT NMR (101 MHz, CDCl_3) δ 142.09 (C), 141.02 (C), 128.86 (CH), 127.19 (CH), 125.18 (CH), 123.01 (C), 121.94 (C), 120.74 (CH), 120.36 (CH), 109.44 (CH), 109.37 (CH), 101.43 (C), 43.35 (CH_2), 29.38 (CH_2), 28.64 (CH_2), 22.48 (CH_2), 13.99 (CH_3). ESI: m/z 263.2 (M + H) $^+$. HRMS calcd for $\text{C}_{18}\text{H}_{19}\text{N}_2$ (M + H) $^+$ 263.1548, found 263.1522.

4.1.25. 9-Pentyl-9H-carbazol-3-yl(piperidin-1-yl)methanethione (25)—Under argon atmosphere, a solution of **4** (60 mg, 0.17 mmol) and LR (49 mg, 0.12 mmol) in toluene (3 mL) was tightly capped in a 5 mL microwave vessel. The mixture was subjected to microwave irradiation at 140°C for 3 h and then cooled to room temperature. The organic solvent was evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel using heptanes/EtOAc in different proportions to yield the target product as a yellow glass. Yield: 48 mg (76%). ^1H NMR (500 MHz, CDCl_3) δ 8.12 – 8.00 (m, 2H), 7.47 (ddd, $J = 8.2, 7.1, 1.1$ Hz, 1H), 7.44 (dd, $J = 8.4, 1.7$ Hz, 1H), 7.40 (d, $J = 8.2$ Hz, 1H), 7.34 (d, $J = 8.4$ Hz, 1H), 7.25 – 7.20 (m, 1H), 4.42 (br. s, 2H), 4.28 (t, $J = 7.2$ Hz, 2H), 3.68 – 3.60 (m, 2H), 1.90 – 1.82 (m, 4H), 1.76 (dt, $J = 11.8, 6.1$ Hz, 2H), 1.62 – 1.56 (m, 2H), 1.35 (dq, $J = 7.2, 3.7$ Hz, 4H), 0.87 (t, $J = 7.0$ Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 201.31 (C=S), 141.09 (C), 140.55 (C), 134.30 (C), 126.23 (CH), 124.23 (CH), 122.84 (C),

122.47 (C), 120.71 (CH), 119.33 (CH), 118.53 (CH), 109.09 (CH), 108.45 (CH), 53.76 (CH₂), 51.46 (CH₂), 43.34 (CH₂), 29.51 (CH₂), 28.83 (CH₂), 27.16 (CH₂), 25.76 (CH₂), 24.44 (CH₂), 22.64 (CH₂), 14.13 (CH₃). ESI: m/z 366.1 (M + H)⁺. HRMS calcd for C₂₃H₂₉N₂S (M + H)⁺ 365.2051, found 365.2019.

4.1.26. 9-Pentyl-3-(piperidin-1-ylmethyl)-9H-carbazole (26)—A solution of **4** (495 mg, 1.42 mmol) in anhydrous tetrahydrofuran (5 mL) was added dropwise to a 0°C cooled suspension of LAH (108 mg, 2.85 mmol) in anhydrous THF (50 mL). The reaction was then heated under reflux for 10 min. The reaction mixture was cooled to 0°C, and then water (3 mL) followed by 3.6 M NaOH solution (70 mL) were carefully added dropwise to the reaction to destroy the excess of LAH. The reaction mixture was then extracted with methyl *tert*-butyl ether (3 × 50 mL). The combined organic layers were washed with brine, dried (MgSO₄) and evaporated. The obtained residue was purified by flash chromatography eluting with heptane/EtOAc (gradient elution) on a Biotage® KP-NH cartridge yielding compound **26** as a light orange oil (263 mg, 55%). ¹H NMR of free base (500 MHz, CDCl₃) δ 8.23 (d, J = 7.7 Hz, 1H), 8.17 (s, 1H), 7.59 – 7.51 (m, 2H), 7.46 (d, J = 8.2 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.33 (t, J = 7.4 Hz, 1H), 4.29 (t, J = 7.2 Hz, 2H), 3.78 (s, 2H), 2.57 (br. s, 4H), 1.92 (p, J = 7.2 Hz, 2H), 1.80 – 1.67 (m, 4H), 1.62 – 1.52 (br. m, 2H), 1.49 – 1.36 (m, 4H), 0.98 (t, J = 7.0 Hz, 3H). ¹³C and APT NMR of free base (101 MHz, CDCl₃) δ 140.71 (C), 139.76 (C), 128.58 (C), 127.39 (CH), 125.49 (CH), 122.79 (C), 122.71 (C), 121.16 (CH), 120.40 (CH), 118.64 (CH), 108.62 (CH), 108.20 (CH), 64.29 (CH₂), 54.47 (CH₂), 43.02 (CH₂), 29.40 (CH₂), 28.71 (CH₂), 25.99 (CH₂), 24.51 (CH₂), 22.50 (CH₂), 13.99 (CH₃). ESI: m/z 335.2 (M + H)⁺. HRMS calcd for C₂₃H₃₁N₂ (M + H)⁺ 335.2487, found 335.2495.

4.1.27. 3-Bromo-9-pentyl-9H-carbazole (27)—Under an argon atmosphere, a mixture of 3-bromo-9H-carbazole (400 mg, 1.63 mmol), 1-bromopentane (0.3 mL, 2.45 mmol) and Cs₂CO₃ (1 g, 3.25 mmol) in DMF (5 mL) was subjected to microwave irradiation at 140°C for 1 h. After cooling to room temperature, the reaction was diluted with EtOAc and filtered. The organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with ethyl acetate/heptanes in different proportions to give 390.4 g (75.9%) of the target product in the form of a yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 8.10 (d, J = 7.77 Hz, 1H) 8.01 (s, 1H) 7.49-7.36 (m, 3H) 7.33 (d, J = 7.83 Hz, 1H) 7.21 (t, J = 7.78 Hz, 1H) 4.29 (q, J = 7.3 Hz, 2H), 1.95-1.84 (m, 2H) 1.42-1.31 (m, 4H) 0.87 (t, J = 6.85 Hz, 3H) ¹³C NMR (126 MHz, CDCl₃) δ 140.72, 139.83, 128.16, 127.25, 125.63, 122.72, 121.25, 120.44, 118.74, 118.60, 108.78, 108.40, 55.15, 29.46, 28.75, 22.55, 13.99.

4.1.28. 3-((4-Methylpiperazin-1-yl)methyl)-9-pentyl-9H-carbazole (28)—Under an argon atmosphere, a mixture of bromide **27** (100 mg, 0.31 mmol), potassium methyl-4-trifluoroboratomethyl-piperazine (75.1 mg, 0.34), K₂CO₃ (131 mg, 0.95 mmol), Pd(OAc)₂ (4.6 mg, 0.015 mmol), and 2-biphenyl-di-*tert*-butylphosphine (12.2 mg, 0.031 mmol) in DMF (2 mL) was subjected to microwave irradiation at 120°C for 20 minutes. The reaction was then cooled to room temperature, diluted with EtOAc, and filtered through a pad of Celite. The filtrate was then washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with ethyl DCM/methanol in different proportions to give 30 mg (28.5%) of the target product in the form of a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 8.09 (d, J = 7.83 Hz, 1H) 8.02 (s, 1H) 7.47-7.38 (m, 3) 7.34 (d, J = 7.82 Hz, 1H) 7.23 (t, J = 7.83 Hz, 1H) 4.28 (q, J = 7.34 Hz, 2H) 3.74 (s, 2H) 2.65-2.58 (m, 4H), 2.56-47 (m, 4H), 1.92-1.85 (m, 2H), 1.62 (s, 9H), 1.42-1.35 (m, 4H), 0.88 (t, J = 6.85 Hz, 3H). ¹³C NMR and DEPT NMR (126 MHz, CDCl₃) δ 140.68 (C), 139.79 (C), 128.12 (CH), 127.21

(C), 125.59 (CH), 122.68 (C), 121.21 (C), 120.40 (C), 118.70 (CH), 118.56 (C), 108.74 (CH), 108.36 (CH), 63.48 (CH₂), 56.04 (CH₂), 55.11 (CH₂), 49.64 (CH₂), 46.61 (CH₂), 29.42 (CH₃), 28.70 (CH₂), 22.50 (CH₂), 13.95 (CH₃). HRMS calcd for C₂₃H₃₂N₃ (M + H)⁺ 350.2596, found 350.2609.

4.1.29. *Tert*-butyl 4-((9-pentyl-9*H*-carbazol-3-yl)methyl)piperazine-1-

carboxylate (29)—Under an argon atmosphere, bromide **27** (100 mg, 0.31), potassium trifluoroboratomethyl -4-*N*-Boc-piperazine (104.1 mg, 0.341 mmol), K₂CO₃ (131 mg, 0.95 mmol), Pd(OAc)₂ (4.6 mg, 0.015 mmol), (2-biphenyl)-di-*tert*-butylphosphine (12.2 mg, 0.031 mmol), and DMF (2 mL) were added to a microwave vessel (10 mL). The reaction was heated in a microwave at 120°C for 20 minutes. The reaction was then cooled to room temperature, diluted with EtOAc, and filtered through a pad of Celite. The filtrate was then washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with DCM/methanol in different proportions to give 85.7 mg (63.5%) of the target product in the form of a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 8.09 (d, *J* = 7.83 Hz, 1H), 8.02(s, 1H), 7.47-7.38 (m, 3H), 7.34 (d, *J* = 7.82 Hz, 1H), 7.23 (t, *J* = 7.77 Hz, 1H), 4.28 (q, *J* = 7.34 Hz, 2H), 3.74 (s, 2H), 2.65-2.58 (m, 4H), 2.56-47 (m, 4H), 1.92-1.85 (m, 2H), 1.62 (s, 9H), 1.42-1.35 (m, 4H), 0.88 (t, *J* = 6.85 Hz, 3H). ¹³C NMR and DEPT NMR (126 MHz, CDCl₃) δ 156.16 (C), 140.40 (C), 139.59 (C), 128.12 (CH), 127.31 (CH), 125.79 (C), 122.68 (CH), 121.21 (C), 120.58 (CH), 118.70 (CH), 118.56 (C), 108.71 (CH), 108.24 (CH), 79.01 (C), 63.49 (CH₂), 55.11 (CH₂), 53.56 (CH₂), 50.52 (CH₂), 29.45 (CH₂), 28.71 (CH₃), 28.46 (CH₂), 22.53 (CH₂), 13.97 (CH₃).

4.1.30. 9-Pentyl-3-(piperazin-1-ylmethyl)-9*H*-carbazole (30)—Compound **29** (60 mg, 0.16 mmol) was added to a solution of DCM (2 mL) and TFA (2 mL). The reaction mixture was stirred for 6 hours at room temperature. The reaction mixture was then diluted with DCM (30 mL), and the organic phase was washed consecutively with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with ethyl acetate/heptanes in different proportions to give 51.2 mg (94.9%) of the target product in the form of a yellow oil. ¹H NMR 8.09 (d, *J* = 7.83 Hz, 1H), 8.02(s, 1H), 7.46(t, *J* = 7.34 Hz, 1H), 7.39 (d, *J* = 7.83 Hz, 2H), 7.34 (d, *J* = 8.31 Hz, 1H), 7.22 (t, *J* = 7.34 Hz, 1H), 4.28 (q, *J* = 7.35 Hz, 2H), 3.72 (s, 2H), 2.89-2.80 (m, 4H), 2.47-2.39 (m, 4H), 1.92-84 (m, 2H), 1.42-1.34 (m, 4H), 0.88 (t, *J* = 6.85 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 140.41 (C), 139.68 (C), 128.12 (CH), 127.31 (C), 125.21 (C), 122.68 (CH), 121.24 (C), 120.44 (CH), 118.77 (C), 118.57 (CH), 108.74 (CH), 108.36 (CH), 63.45 (CH₂), 57.53 (CH₂), 55.11 (CH₂), 45.92 (CH₂), 29.37 (CH₂), 28.72 (CH₂), 22.51 (CH₂), 13.94 (CH₃). HRMS calcd for C₂₂H₃₀N₃ (M + H)⁺ 336.2440, found 336.2466.

4.1.31. Ethyl 9-pentyl-9*H*-pyrido[3,4-*b*]indole-3-carboxylate (31)—Using ethyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate (1 g, 4.16 mmol) and *n*-bromopentane (772 μL, 6.24 mmol) as starting compounds, the title compound was prepared as a white solid according to the procedure described above for compound **1** using method A. The target product was purified using heptanes/EtOAc in different proportions to afford the title compound as a white solid (1.08 g, 84%); mp 92°C. ¹H NMR (500 MHz, CDCl₃) δ 8.91 (s, 1H), 8.82 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.59 (ddd, *J* = 8.4, 7.1, 1.2 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.31 (t, *J* = 7.5 Hz, 1H), 4.55 (q, *J* = 7.1 Hz, 2H), 4.32 (t, *J* = 7.1 Hz, 2H), 1.97 – 1.76 (m, 2H), 1.51 (t, *J* = 7.1 Hz, 3H), 1.39 – 1.21 (m, 4H), 0.83 (t, *J* = 6.7 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 166.07 (C=O), 141.31 (C), 137.59 (C), 137.39 (C), 131.54 (CH), 128.64 (CH), 128.07 (C), 121.90 (CH), 121.16 (C), 120.40 (CH), 117.49 (CH), 109.77 (CH), 61.40 (CH₂), 43.41 (CH₂), 29.16 (CH₂), 28.75 (CH₂), 22.25 (CH₂), 14.48 (CH₃), 13.77

(CH₃). ESI: m/z 311.1 (M + H)⁺. HRMS calcd for C₁₉H₂₃N₂O₂ (M + H)⁺ 311.1760, found 311.1784.

4.1.32. 9-Pentyl-9H-pyrido[3,4-b]indole-3-carboxylic acid (32)—Potassium hydroxide (3 g, 53.57 mmol) was added to a stirred solution of ester **31** (715 mg, 2.30 mmol) in a mixture of ethanol (40 mL) and water (10 mL). The reaction mixture was stirred at reflux for 16 hrs and then cooled to room temperature. The solvents were evaporated under reduced pressure, and the residue was diluted with DI water. Using external cooling (ice-bath), the solution was acidified to pH *ca.* 2 by dropwise addition of 1M aqueous HCl. The precipitated product was extracted with EtOAc (150 mL). The organic layer was washed with brine under acidic pH, dried over MgSO₄, and filtered. The solvent was then evaporated under reduced pressure, and the resulting residue was purified by silica chromatography using EtOAc/heptane in different proportions to yield the title compound as a pinkish solid. Yield: 647 mg (100%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.56 (br. s, 1H), 9.49 (s, 1H), 9.33 (s, 1H), 8.66 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.84 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 4.72 (t, *J* = 7.1 Hz, 2H), 1.88 – 1.71 (m, 2H), 1.34 – 1.21 (m, 4H), 0.79 (t, *J* = 6.9 Hz, 3H). ¹³C and APT NMR (101 MHz, DMSO-*d*₆) δ 162.53 (C=O), 143.52 (C), 136.37 (C), 131.58 (CH), 131.36 (C), 130.41 (C), 127.97 (CH), 123.83 (CH), 122.05 (CH), 120.08 (C), 118.89 (CH), 111.57 (CH), 43.63 (CH₂), 28.58 (CH₂), 28.35 (CH₂), 21.85 (CH₂), 13.81 (CH₃).

4.1.33. 1-((9-Pentyl-9H-pyrido[3,4-b]indol-3-yl)carbonyl)piperidine (33)—Using acid **32** (241 mg, 0.85 mmol) and piperidine (102 mg, 1.20 mmol) as starting compounds, the title compound was prepared as an off-white solid according to the analogous procedure described above for **4** by the amide coupling protocol. Yield: 122 mg (41%); mp 126°C. ¹H NMR (400 MHz, CDCl₃) δ 8.81 (s, 1H), 8.38 (s, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 7.59 (ddd, *J* = 8.4, 7.1, 1.3 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.29 (t, *J* = 7.5 Hz, 1H), 4.36 (t, *J* = 7.2 Hz, 2H), 3.81 (br. s, 2H), 3.63 (br. s, 2H), 1.89 (p, *J* = 7.1 Hz, 2H), 1.79 – 1.66 (m, 4H), 1.60 (br. s, 2H), 1.39 – 1.28 (m, 4H), 0.85 (t, *J* = 6.9 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 168.86 (C=O), 143.75 (C), 141.56 (C), 136.45 (C), 129.95 (CH), 128.80 (C), 128.68 (CH), 122.09 (CH), 121.22 (C), 120.06 (CH), 115.60 (CH), 109.72 (CH), 48.71 (CH₂), 43.77 (CH₂), 43.54 (CH₂), 29.31 (CH₂), 28.87 (CH₂), 26.70 (CH₂), 25.73 (CH₂), 24.75 (CH₂), 22.40 (CH₂), 13.92 (CH₃). ESI: m/z 350.2 (M + H)⁺. HRMS calcd for C₂₂H₂₈N₃O (M + H)⁺ 350.2232, found 350.2249.

4.1.34. 9-Pentyl-N-(piperidin-1-yl)-9H-pyrido[3,4-b]indole-3-carboxamide (34)—Using acid **32** (100 mg, 0.35 mmol) and 1-aminopiperidine (36 mg, 0.36 mmol) as starting compounds, the title compound was prepared as a light yellow glass according to the analogous procedure described above for **4** by the amide coupling protocol with the exception of not using 5% citric acid solution during the workup. Yield: 71 mg (56%). ¹H NMR (500 MHz, CDCl₃) δ 8.93 (s, 1H), 8.81 (s, 1H), 8.71 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 4.36 (t, *J* = 7.1 Hz, 2H), 2.93 (br. s, 4H), 1.94 – 1.85 (m, 2H), 1.82 (dt, *J* = 10.9, 5.4 Hz, 4H), 1.49 (br. s, 2H), 1.39 – 1.28 (m, 4H), 0.86 (t, *J* = 6.9 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 162.31 (C=O), 141.59 (C), 139.89 (C), 137.77 (C), 129.82 (CH), 128.96 (C), 128.81 (CH), 122.46 (CH), 121.58 (C), 120.48 (CH), 114.90 (CH), 109.82 (CH), 57.49 (CH₂), 43.73 (CH₂), 29.42 (CH₂), 29.00 (CH₂), 25.41 (CH₂), 23.55 (CH₂), 22.51 (CH₂), 14.02 (CH₃). ESI: m/z 365.1 (M + H)⁺. HRMS calcd for C₂₂H₂₉N₄O (M + H)⁺ 365.2341, found 365.2316.

4.1.35. N-(2,2-dimethylpropyl)-9-pentyl-9H-pyrido[3,4-b]indole-3-carboxamide (35)—Using acid **32** (100 mg, 0.35 mmol) and neopentylamine (37 mg, 0.42 mmol) as

starting compounds, the title compound was prepared as a white solid according to the analogous procedure described above for **4** by the amide coupling protocol. Yield: 67 mg (54%); mp 91°C. ¹H NMR (500 MHz, CDCl₃) δ 8.93 (d, *J* = 0.9 Hz, 1H), 8.74 (d, *J* = 0.9 Hz, 1H), 8.33 (s, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 7.60 (td, *J* = 7.2, 3.6 Hz, 1H), 7.46 (d, *J* = 8.3 Hz, 1H), 7.32 (ddd, *J* = 8.0, 7.1, 0.9 Hz, 1H), 4.36 (t, *J* = 7.2 Hz, 2H), 3.36 (d, *J* = 6.6 Hz, 2H), 2.02 – 1.78 (m, 2H), 1.45 – 1.27 (m, 4H), 1.04 (s, 9H), 0.87 (t, *J* = 7.0 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 165.53 (C=O), 141.53 (C), 140.07 (C), 137.61 (C), 129.84 (CH), 128.91 (C), 128.61 (CH), 122.24 (CH), 121.52 (C), 120.28 (CH), 114.38 (CH), 109.68 (CH), 50.67 (CH₂), 43.60 (CH₂), 32.32 (C), 29.31 (CH₂), 28.89 (CH₂), 27.39 (CH₃), 22.39 (CH₂), 13.91 (CH₃). ESI: *m/z* 352.2 (M + H)⁺. HRMS calcd for C₂₂H₃₀N₃O (M + H)⁺ 352.2389, found 352.2386.

4.1.36. 9-Propyl-9H-carbazole (36)—From carbazole (2 g, 11.96 mmol), *n*-propyl iodide (1.75 mL, 17.91 mmol), and NaOH (718 mg, 17.95 mmol) a similar procedure as that described compound **1** (*Method B*) afforded the title product as a white waxy solid. Yield: 2.07 g (83%). ¹H NMR (400 MHz, CDCl₃) δ 8.09 (d, *J* = 7.8 Hz, 2H), 7.44 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.21 (ddd, *J* = 8.0, 6.8, 1.1 Hz, 2H), 4.24 (t, *J* = 7.2 Hz, 2H), 2.01 – 1.78 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 140.71 (C), 125.75 (CH), 123.00 (C), 120.53 (CH), 118.90 (CH), 108.89 (CH), 44.80 (CH₂), 22.51 (CH₂), 12.02 (CH₃).

4.1.37. 9-Butyl-9H-carbazole (37)—From carbazole (10 g, 59.81 mmol) and *n*-butyl iodide (10 mL, 87.87 mmol), a similar procedure as that described for compound **1** (*Method B*) afforded the title product as a white solid. Yield: 10.53 g (79%); mp 62°C. ¹H NMR (500 MHz, CDCl₃) δ 8.09 (d, *J* = 7.7 Hz, 2H), 7.45 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.21 (*J* = 7.02, 6.83, 1.1 Hz, 1H), 4.27 (t, *J* = 7.2 Hz, 2H), 1.89 – 1.76 (m, 2H), 1.44 – 1.32 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 140.65 (C), 125.75 (CH), 123.02 (C), 120.53 (CH), 118.89 (CH), 108.85 (CH), 43.02 (CH₂), 31.33 (CH₂), 20.78 (CH₂), 14.09 (CH₃).

4.1.38. 9-Hexyl-9H-carbazole (38)—A mixture of carbazole (1.5 g, 8.97 mmol), *n*-hexyl bromide (1.89 mL, 13.46 mmol), and powdered NaOH (538 mg, 13.45 mmol) in dry acetone (50 mL) was refluxed for 16 h under nitrogen. After all volatile components were removed by rotary evaporation *in vacuo*, the residue was extracted with *tert*-butyl methyl ether (100 mL). The organic phase was washed with water, brine, dried (MgSO₄), filtered, and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a colorless oil which crystallized on standing (2.03 g, 90%); mp 66°C. ¹H NMR (500 MHz, CDCl₃) δ 8.10 (d, *J* = 7.7 Hz, 2H), 7.45 (t, *J* = 7.3 Hz, 2H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.22 (t, *J* = 7.6 Hz, 2H), 4.28 (t, *J* = 7.3 Hz, 2H), 1.86 (dt, *J* = 15.0, 7.5 Hz, 2H), 1.43 – 1.34 (m, 2H), 1.34 – 1.21 (m, 4H), 0.86 (t, *J* = 7.0 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 140.61 (C), 125.75 (CH), 122.99 (C), 120.53 (CH), 118.87 (CH), 108.84 (CH), 43.28 (CH₂), 31.81 (CH₂), 29.15 (CH₂), 27.20 (CH₂), 22.77 (CH₂), 14.25 (CH₃).

4.1.39. 9-Heptyl-9H-carbazole (39)—From carbazole (2 g, 11.96 mmol), *n*-heptyl bromide (3.21 g, 17.94 mmol), and NaOH (4 g, 100 mmol), an analogous procedure as that described for compound **38** afforded the target product as a colorless oil. Yield 2.88 g (91%). ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, *J* = 7.8 Hz, 2H), 7.42 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 2H), 7.34 (d, *J* = 8.2 Hz, 2H), 7.19 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 2H), 4.19 (t, *J* = 7.3 Hz, 2H), 1.86 – 1.73 (m, 2H), 1.38 – 1.13 (m, 8H), 0.84 (t, *J* = 6.9 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 140.62 (C), 125.73 (CH), 123.01 (C), 120.51 (CH), 118.87 (CH),

108.83 (CH), 43.20 (CH₂), 31.92 (CH₂), 29.28 (CH₂), 29.16 (CH₂), 27.46 (CH₂), 22.79 (CH₂), 14.25 (CH₃).

4.1.40. Piperidin-1-yl(9-propyl-9H-carbazol-3-yl)methanone (40)—Using 9-propyl-9H-carbazole (1.484 g, 7.09 mmol), piperidine-1-carbonyl chloride (1.064 mL, 8.51 mmol), and anhydrous AlCl₃ (1.04 g, 7.80 mmol) as starting compounds, the title compound was prepared as a white crystalline solid according to the procedure described above for **4** (*Method B: Friedel–Crafts acylation*). Yield: 1.720 g (76%); mp 149°C. ¹H NMR (400 MHz, CDCl₃) δ 8.19 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.39 (t, *J* = 8.6 Hz, 2H), 7.23 (t, *J* = 7.3 Hz, 1H), 4.25 (t, *J* = 7.0 Hz, 2H), 3.62 (br. s, 4H), 1.98 – 1.82 (m, 2H), 1.77 – 1.48 (br. m, 6H), 0.94 (t, *J* = 7.4 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 171.60 (C=O), 141.15 (C), 141.06 (C), 126.87 (C), 126.19 (CH), 125.13 (CH), 122.82 (C), 122.51 (C), 120.60 (CH), 119.93 (CH), 119.36 (CH), 109.11 (CH), 108.50 (CH), 44.83 (CH₂), 26.33 (CH₂), 24.86 (CH₂), 22.40 (CH₂), 11.89 (CH₃). ESI: *m/z* 321.1 (M + H)⁺. HRMS calcd for C₂₁H₂₅N₂O (M + H)⁺ 321.1967, found 321.1934.

4.1.41. (9-Hexyl-9H-carbazol-3-yl)(piperidin-1-yl)methanone (41)—From 9-hexyl-9H-carbazole (1.651 g, 6.57 mmol), piperidine-1-carbonyl chloride (986 μL, 7.88 mmol), and anhydrous AlCl₃ (963 mg, 7.22 mmol) an analogous microwave procedure as that described for **4** (*Method B: Friedel–Crafts acylation*) afforded the title product as a light yellow gum. Yield 1.77 g (74%). ¹H NMR (500 MHz, CDCl₃) δ 8.19 (d, *J* = 1.2 Hz, 1H), 8.09 (d, *J* = 7.6 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.47 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.24 (ddd, *J* = 7.9, 7.0, 0.9 Hz, 1H), 4.28 (t, *J* = 7.3 Hz, 2H), 3.62 (br. s, 4H), 1.85 (dt, *J* = 15.0, 7.4 Hz, 2H), 1.76 – 1.67 (br. m, 3H), 1.63 (br. s, 3H), 1.41 – 1.32 (m, 2H), 1.32 – 1.21 (m, 4H), 0.85 (t, *J* = 7.1 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 171.53 (C=O), 140.99 (C), 140.90 (C), 126.76 (C), 126.11 (CH), 125.06 (CH), 122.75 (C), 122.43 (C), 120.53 (CH), 119.86 (CH), 119.26 (CH), 108.98 (CH), 108.37 (CH), 43.24 (CH₂), 31.59 (CH₂), 28.94 (CH₂), 26.96 (CH₂), 26.25 (CH₂), 24.79 (CH₂), 22.56 (CH₂), 14.04 (CH₃). ESI: *m/z* 363.2 (M + H)⁺. HRMS calcd for C₂₄H₃₀N₂O (M + H)⁺ 363.2436, found 363.2423.

4.1.42. 9-Heptyl-9H-carbazol-3-yl)(piperidin-1-yl)methanone (42)—Using 9-heptyl-9H-carbazole (1.743 g, 6.57 mmol), piperidine-1-carbonyl chloride (986 μL, 7.88 mmol), and anhydrous AlCl₃ (963 mg, 7.22 mmol) as starting compounds, the title compound was prepared as a light yellow gum according to the procedure described above for **4** (*Method B: Friedel–Crafts acylation*). Yield: 1.737 g (70%). ¹H NMR (400 MHz, CDCl₃) δ 8.18 (d, *J* = 1.2 Hz, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.48 (ddd, *J* = 8.2, 7.1, 1.1 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 1H), 7.24 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 4.29 (t, *J* = 7.2 Hz, 2H), 3.62 (br. s, 4H), 1.92 – 1.79 (m, 2H), 1.76 – 1.66 (br. m, 3H), 1.63 (br. s, 3H), 1.41 – 1.28 (m, 4H), 1.28 – 1.17 (m, 4H), 0.85 (t, *J* = 6.9 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 171.67 (C=O), 141.13 (C), 141.04 (C), 126.89 (C), 126.23 (CH), 125.20 (CH), 122.89 (C), 122.57 (C), 120.67 (CH), 119.99 (CH), 119.40 (CH), 109.11 (CH), 108.50 (CH), 43.38 (CH₂), 31.86 (CH₂), 29.21 (CH₂), 29.12 (CH₂), 27.40 (CH₂), 26.37 (CH₂), 24.92 (CH₂), 22.73 (CH₂), 14.20 (CH₃). ESI: *m/z* 377.3 (M + H)⁺. HRMS calcd for C₂₅H₃₂N₂O (M + H)⁺ 377.2593, found 377.2554.

4.1.43. Ethyl 3-(9H-carbazol-9-yl)propanoate (43)—The title compound was prepared according to a procedure already described. (*J. Med. Chem.* 50, 4648–4655, 2007). ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, *J* = 7.8 Hz, 2H), 7.48 – 7.39 (m, 4H), 7.22 (ddd, *J* = 8.0, 6.4, 1.8 Hz, 2H), 4.61 (t, *J* = 7.2 Hz, 2H), 4.06 (q, *J* = 7.2 Hz, 2H), 2.81 (t, *J* = 7.2 Hz, 2H), 1.13 (t, *J* = 7.2 Hz, 3H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 171.59

(C=O), 140.17 (C), 125.95 (CH), 123.26 (C), 120.56 (CH), 119.35 (CH), 108.80 (CH), 61.06 (CH₂), 38.90 (CH₂), 33.73 (CH₂), 14.19 (CH₃).

4.1.44. 2-(9H-carbazol-9-yl)ethanol (44)—To a stirred solution of carbazole (3 g, 17.94 mmol) in methyl ethyl ketone (10 mL) in a 20 mL microwave vessel, powdered KOH (1 g, 17.82 mmol) was added under nitrogen, and the solution was cooled to -60°C . Ice-cold ethylene oxide (8 mL, 2.5 M solution in THF) was added dropwise to the obtained solution, and the reaction vessel was tightly capped. The reaction mixture was then subjected to microwave irradiation at 50°C for 1 h with stirring and then cooled to room temperature. The solution was extracted with *tert*-butyl methyl ether (150 mL). The organic phase was washed with water, hydrochloric acid (aq, 2 M), brine, dried (MgSO₄), and filtered. The organic solvents were evaporated *in vacuo*, and the obtained residue self-crystallized. The crystalline product was removed by vacuum filtration, washed with hexanes (20 mL), and then dried under high vacuum for 12 h to give the title product as a white solid, which was used without further purification. Yield: 3.136 g (83%); mp 83°C . ¹H NMR (500 MHz, CDCl₃) δ 7.99 (d, $J = 7.7$ Hz, 2H), 7.37 (ddd, $J = 8.2, 7.2, 1.2$ Hz, 2H), 7.28 (d, $J = 8.2$ Hz, 2H), 7.16 (ddd, $J = 7.9, 7.0, 0.9$ Hz, 2H), 4.19 (t, $J = 5.4$ Hz, 2H), 3.71 (t, $J = 5.4$ Hz, 2H), 1.82 (s, 1H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 140.77 (C), 125.93 (CH), 123.01 (C), 120.46 (CH), 119.30 (CH), 108.95 (CH), 61.38 (CH₂), 45.46 (CH₂).

4.1.45. 3-(9H-carbazol-9-yl)propan-1-ol (45)—The title compound was prepared according to a procedure already described[22]. ¹H NMR (500 MHz, CDCl₃) δ 8.06 (d, $J = 7.8$ Hz, 2H), 7.46 – 7.35 (m, 4H), 7.20 (ddd, $J = 7.9, 6.6, 1.5$ Hz, 2H), 4.34 (t, $J = 6.6$ Hz, 2H), 3.47 (t, $J = 5.9$ Hz, 2H), 1.99 (p, $J = 6.3$ Hz, 2H), 1.72 (br. s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 140.57 (C), 125.83 (CH), 122.94 (C), 120.49 (CH), 119.02 (CH), 108.79 (CH), 59.67 (CH₂), 39.29 (CH₂), 31.50 (CH₂).

4.1.46. 9-Butyl-9H-carbazole-3-carbaldehyde (46)—Using 9-butyl-9H-carbazole (2.982 g, 13.35 mmol), DMF (7.43 mL, 95.96 mmol), and POC1₃ (2.80 mL, 30.04 mmol) as starting compounds, the title compound was prepared as yellowish crystals following the procedures described in preparation of compound 2. Yield: 3.185 g (quantitative); mp 58°C . ¹H NMR (400 MHz, CDCl₃) δ 10.05 (s, 1H), 8.55 (s, 1H), 8.11 (d, $J = 7.7$ Hz, 1H), 7.96 (d, $J = 8.5$ Hz, 1H), 7.50 (t, $J = 7.6$ Hz, 1H), 7.41 (d, $J = 8.6$ Hz, 2H), 7.30 (t, $J = 7.4$ Hz, 1H), 4.26 (t, $J = 7.1$ Hz, 2H), 1.91 – 1.73 (m, 2H), 1.46 – 1.28 (m, 2H), 0.93 (t, $J = 7.3$ Hz, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 191.88 (C=O), 144.16 (C), 141.28 (C), 128.58 (C), 127.22 (CH), 126.82 (CH), 124.06 (CH), 123.14 (C), 123.08 (C), 120.84 (CH), 120.40 (CH), 109.53 (CH), 109.05 (CH), 43.26 (CH₂), 31.16 (CH₂), 20.63 (CH₂), 13.97 (CH₃).

4.1.47. Ethyl 3-(3-formyl-9H-carbazol-9-yl)propanoate (47)—Using ethyl 3-(9H-carbazol-9-yl)propanoate (5.012 g, 18.75 mmol), DMF (10 mL, 129.16 mmol), and POC1₃ (3.5 mL, 37.44 mmol) as starting compounds, the title compound was prepared as white crystals following the procedures described in preparation of compound 2. Yield: 3.642 g (66%); mp 79°C . ¹H NMR (500 MHz, CDCl₃) δ 10.06 (s, 1H), 8.55 (d, $J = 1.0$ Hz, 1H), 8.11 (d, $J = 7.8$ Hz, 1H), 7.98 (dd, $J = 8.5, 1.5$ Hz, 1H), 7.52 (ddd, $J = 8.2, 7.0, 1.2$ Hz, 1H), 7.51 (d, $J = 8.5$ Hz, 1H), 7.47 (d, $J = 8.2$ Hz, 1H), 7.32 (ddd, $J = 7.9, 6.8, 1.1$ Hz, 1H), 4.63 (t, $J = 7.0$ Hz, 2H), 4.07 (q, $J = 7.2$ Hz, 2H), 2.85 (t, $J = 7.0$ Hz, 2H), 1.13 (t, $J = 7.2$ Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 191.88 (HC=O), 171.17 (C=O), 143.79 (C), 140.79 (C), 128.93 (C), 127.46 (CH), 127.01 (CH), 123.89 (CH), 123.38 (C), 123.25 (C), 120.91 (CH), 120.78 (CH), 109.46 (CH), 109.17 (CH), 61.23 (CH₂), 39.12 (CH₂), 33.60 (CH₂), 14.15 (CH₃).

4.1.48. 9-(2-Chloroethyl)-9H-carbazole-3-carbaldehyde (48)—Using compound **44** (2.86 g, 13.54 mmol), DMF (9 mL, 116.24 mmol), and POCl_3 (3.37 mL, 36.16 mmol) as starting compounds, the title compound was prepared as a white-tan solid following the procedures described in preparation of compound **2**. Yield: 2.826 g (81%); mp 167°C. ^1H NMR (400 MHz, CDCl_3) δ 10.08 (s, 1H), 8.58 (s, 1H), 8.14 (d, $J = 7.8$ Hz, 1H), 8.00 (d, $J = 8.5$ Hz, 1H), 7.54 (t, $J = 7.8$ Hz, 1H), 7.50 (d, $J = 8.6$ Hz, 1H), 7.46 (d, $J = 8.2$ Hz, 1H), 7.35 (t, $J = 7.4$ Hz, 1H), 4.66 (t, $J = 6.7$ Hz, 2H), 3.89 (t, $J = 6.7$ Hz, 2H). ^{13}C NMR (101 MHz, CDCl_3) δ 191.86 (CHO), 144.10 (C), 141.00 (C), 129.32 (C), 127.65 (CH), 127.21 (CH), 124.02 (CH), 123.56 (C), 123.36 (C), 121.13 (CH), 121.10 (CH), 109.33 (CH), 109.14 (CH), 45.13 (CH_2), 41.29 (CH_2).

4.1.49. 9-(3-Chloropropyl)-9H-carbazole-3-carbaldehyde (49)—The title compound was prepared according to a modified literature procedure[34]. POCl_3 (2.8 mL, 30.04 mmol) was added, over a period of 10 min, to an ice-cooled, stirred DMF (7.43 mL, 95.96 mmol) under nitrogen. The reddish solution was allowed to stir at room temperature for 1 h. Compound **45** (2.447 g, 10.86 mmol) was added over 10 min, and the obtained mixture was subjected to microwave irradiation at 100°C for 1 h. The reaction mixture was cooled and then poured into crushed ice. After warming to room temperature, the resultant product was extracted with DCM. The organic phase was washed with water, brine, dried (MgSO_4), filtered, and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel using heptanes/EtOAc in different proportions to afford the title compound as a light yellow solid (2.436 g, 83%); mp 88°C. ^1H NMR (500 MHz, CDCl_3) δ 10.05 (s, 1H), 8.54 (s, 1H), 8.11 (d, $J = 7.7$ Hz, 1H), 7.97 (d, $J = 8.4$ Hz, 1H), 7.61 – 7.41 (m, 3H), 7.32 (t, $J = 7.3$ Hz, 1H), 4.48 (t, $J = 6.5$ Hz, 2H), 3.49 (t, $J = 5.8$ Hz, 2H), 2.45 – 2.19 (m, 2H). ^{13}C and APT NMR (126 MHz, CDCl_3) δ 191.84 (C=O), 144.07 (C), 141.11 (C), 128.86 (C), 127.52 (CH), 127.06 (CH), 123.90 (CH), 123.26 (C), 123.15 (C), 120.92 (CH), 120.74 (CH), 109.39 (CH), 108.96 (CH), 42.15 (CH_2), 40.15 (CH_2), 31.68 (CH_2).

4.1.50. 9-Butyl-9H-carbazole-3-carboxylic acid (50)—Powdered potassium permanganate (3 g, 18.98 mmol) was added in one portion to a solution of 9-butyl-9H-carbazole-3-carbaldehyde (2.936 g, 11.68 mmol) in a mixture of acetone (200 mL) and water (3 mL) at room temperature. The resulting solution was stirred for 5 min at this temperature, and then, the mixture was heated 16 h at reflux under stirring. The mixture was quenched with ethanol (20 mL), and then stirred for 30 min at reflux. After cooling, the solid precipitate (MnO_2) was filtered off through a pad of Celite®; the filtrate was concentrated *in vacuo* to remove organic solvents. The obtained syrup was diluted with water (100 mL), basified with NaOH to pH *ca.* 10, and extracted with ether (50 mL \times 3) to remove the unreacted starting material. The aqueous solution was cooled on an ice-water bath and acidified with ice-cold solution of sulfuric acid (20%) to pH *ca.* 2. The resultant bulky precipitate was extracted with EtOAc and the extract was washed with brine, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The precipitated product was collected by filtration, washed with heptanes (20 mL \times 2), and dried overnight to produce the title compound (1.993 g, 64%) as a white solid; mp 159°C. ^1H NMR (500 MHz, CDCl_3) δ 12.82 (s, 1H), 8.91 (d, $J = 1.6$ Hz, 1H), 8.25 (dd, $J = 8.6, 1.6$ Hz, 1H), 8.15 (d, $J = 7.7$ Hz, 1H), 7.50 (ddd, $J = 8.3, 7.1, 1.3$ Hz, 1H), 7.41 (d, $J = 8.2$ Hz, 1H), 7.38 (d, $J = 8.7$ Hz, 1H), 7.29 (ddd, 7.9, 6.9, 1 Hz, 1H), 4.27 (t, $J = 7.2$ Hz, 2H), 1.92 – 1.77 (m, 2H), 1.46 – 1.31 (m, 2H), 0.94 (t, $J = 7.4$ Hz, 3H). ^{13}C and APT NMR (126 MHz, CDCl_3) δ 173.60 (C=O), 143.80 (C), 141.22 (C), 128.03 (CH), 126.59 (CH), 123.90 (CH), 123.14 (C), 122.84 (C), 120.91 (CH), 120.17 (CH), 119.83 (C), 109.36 (CH), 108.44 (CH), 43.25 (CH_2), 31.22 (CH_2), 20.72 (CH_2), 14.05 (CH_3).

4.1.51. 9-(3-Ethoxy-3-oxopropyl)-9H-carbazole-3-carboxylic acid (51)—

Powdered potassium permanganate (736 mg, 4.66 mmol) was added in portions under stirring at room temperature to a solution of **47** (747 mg, 2.53 mmol) in a mixture of acetone (50 mL) and acetic acid (805 μ L). After the addition, the mixture was heated 5 h at reflux under stirring and then allowed to cool to room temperature. The mixture was filtered through a pad of Celite[®] and concentrated *in vacuo* to remove organic solvents. The obtained residue was purified by column chromatography on silica gel, eluting with EtOAc/heptanes in different proportions to give the title compound (434 mg, 55%) as yellow crystalline; mp 153°C. ¹H NMR (500 MHz, CDCl₃) δ 12.69 (br. s, 1H), 8.88 (d, *J* = 1.4 Hz, 1H), 8.26 (dd, *J* = 8.6, 1.6 Hz, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.52 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 2H), 7.33 (ddd, *J* = 7.9, 6.9, 1.0 Hz, 1H), 4.63 (t, *J* = 7.1 Hz, 2H), 4.08 (q, *J* = 7.2 Hz, 2H), 2.86 (t, *J* = 7.1 Hz, 2H), 1.14 (t, *J* = 7.2 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 173.18 (C=O), 171.35 (C=O), 143.44 (C), 140.80 (C), 128.22 (CH), 126.82 (CH), 123.87 (CH), 123.38 (C), 123.15 (C), 120.99 (CH), 120.60 (CH), 120.41 (C), 109.31 (CH), 108.54 (CH), 61.27 (CH₂), 39.16 (CH₂), 33.72 (CH₂), 14.21 (CH₃).

4.1.52. 9-(2-Chloroethyl)-9H-carbazole-3-carboxylic acid (52)—From compound **48** (2.826 g, 10.97 mmol), potassium permanganate (2.08 g, 13.16 mmol), DI water (2 mL, 111.11 mmol), and acetone (100 mL), a similar procedure as that described for compound **50** afforded the title product as a yellow crystalline solid. Yield 2.422 g (81%); mp 204°C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.74 (s, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.23 (t, *J* = 7.4 Hz, 1H), 4.78 (t, *J* = 5.6 Hz, 2H), 4.06 (t, *J* = 5.7 Hz, 2H), 1.87 (s, 1H). ¹³C and APT NMR (101 MHz, DMSO-*d*₆) δ 169.70 (C=O), 141.27 (C), 140.54 (C), 129.48 (C), 127.51 (CH), 125.64 (CH), 122.77 (C), 121.51 (C and CH), 120.20 (CH), 119.33 (CH), 109.65 (CH), 108.13 (CH), 44.19 (CH₂), 43.06 (CH₂).

4.1.53. 9-(3-Chloropropyl)-9H-carbazole-3-carboxylic acid (53)—From 9-(3-chloropropyl)-9H-carbazole-3-carbaldehyde (2.436 g, 8.96 mmol), potassium permanganate (2 g, 12.66 mmol), DI water (2 mL, 111.11 mmol), and acetone (100 mL), a similar procedure as that described for compound **50** afforded the title product as a yellowish solid. Yield 1.605 g (62%); mp 218°C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 12.68 (s, 1H), 8.82 (d, *J* = 1.5 Hz, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.09 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.65 (d, *J* = 8.3 Hz, 1H), 7.51 (ddd, *J* = 8.3, 7.1, 1.3 Hz, 1H), 7.27 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 4.54 (t, *J* = 6.9 Hz, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 2.22 (p, *J* = 6.5 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 168.02 (C=O), 142.48 (C), 140.57 (C), 127.19 (CH), 126.54 (CH), 122.57 (CH), 122.31 (C), 121.99 (C), 121.42 (C), 120.82 (CH), 119.91 (CH), 109.56 (CH), 108.83 (CH), 42.63 (CH₂), 39.90 (CH₂), 31.46 (CH₂).

4.1.54. (9-Butyl-9H-carbazol-3-yl)(piperidin-1-yl)methanone (54)—Using carboxylic acid **50** (400 mg, 1.50 mmol) and piperidine (177 μ L, 1.79 mmol) as starting compounds, the title compound was prepared as a colorless viscous gum according to the procedure described above for compound **4**. Yield: 325 mg (65%). ¹H NMR (500 MHz, CDCl₃) δ 8.09 (d, *J* = 1.2 Hz, 1H), 7.97 (d, *J* = 7.7 Hz, 1H), 7.42 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.35 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H), 7.27 (t, *J* = 8.6 Hz, 2H), 7.11 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 4.14 (t, *J* = 7.1 Hz, 2H), 3.51 (br. s, 4H), 1.70 (dt, *J* = 15.0, 7.3 Hz, 2H), 1.62 – 1.53 (br. m, 3H), 1.50 (br. s, 3H), 1.30 – 1.18 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 171.48 (C=O), 140.94 (C), 140.86 (C), 126.69 (C), 126.08 (CH), 125.02 (CH), 122.68 (C), 122.38 (C), 120.48 (CH), 119.83 (CH), 119.24 (CH), 108.99 (CH), 108.37 (CH), 42.91 (CH₂), 31.08 (CH₂), 24.74 (CH₂), 20.52 (CH₂), 13.90 (CH₃). ESI: *m/z* 335.3 (M + H)⁺. HRMS calcd for C₂₂H₂₇N₂O (M + H)⁺ 335.2123, found 335.2086.

4.1.55. Ethyl 3-(3-(piperidine-1-carbonyl)-9H-carbazol-9-yl)propanoate (55)—

Using carboxylic acid **51** (200 mg, 0.64 mmol) and piperidine (76 μ L, 0.78 mmol) as starting compounds, the title compound was prepared as a colorless gum according to the procedure described above for compound **4**. Yield: 175 mg (72%). ^1H NMR (500 MHz, CDCl_3) δ 8.18 (d, $J = 1.2$ Hz, 1H), 8.06 (d, $J = 7.7$ Hz, 1H), 7.53 (dd, $J = 8.4, 1.5$ Hz, 1H), 7.48 – 7.39 (m, 3H), 7.23 (ddd, $J = 8.0, 6.5, 1.5$ Hz, 1H), 4.60 (t, $J = 7.0$ Hz, 2H), 4.04 (q, $J = 7.2$ Hz, 2H), 3.66 (br. s, 2H), 3.55 (br. s, 2H), 2.81 (t, $J = 7.0$ Hz, 2H), 1.77 – 1.64 (m, 3H), 1.61 (br. s, 3H), 1.11 (t, $J = 7.2$ Hz, 3H). ^{13}C and APT NMR (126 MHz, CDCl_3) δ 171.24 (C=O), 171.18 (C=O), 140.45 (C), 140.37 (C), 127.16 (C), 126.23 (CH), 125.06 (CH), 122.86 (C), 122.65 (C), 120.48 (CH), 119.80 (CH), 119.63 (CH), 108.91 (CH), 108.35 (CH), 60.87 (CH_2), 38.79 (CH_2), 33.48 (CH_2), 24.66 (CH_2), 13.97 (CH_3). ESI: m/z 379.3 (M + H) $^+$. HRMS calcd for $\text{C}_{23}\text{H}_{27}\text{N}_2\text{O}_3$ (M + H) $^+$ 379.2022, found 379.2017.

4.1.56. (9-(2-Chloroethyl)-9H-carbazol-3-yl)(piperidin-1-yl)methanone (56)—

From carboxylic acid **52** (2.2 g, 8.04 mmol) and piperidine (953 μ L, 9.64 mmol), a similar procedure as that described for compound **4** gave the title compound (2.144 g, 78%) as a light yellow crystalline solid; mp 172°C. ^1H NMR (400 MHz, CDCl_3) δ 8.17 (s, 1H), 8.07 (d, $J = 7.8$ Hz, 1H), 7.52 (d, $J = 8.4$ Hz, 1H), 7.47 (t, $J = 7.6$ Hz, 1H), 7.40 (dd, $J = 8.2, 4.2$ Hz, 2H), 7.26 (t, $J = 7.3$ Hz, 1H), 4.60 (t, $J = 6.8$ Hz, 2H), 3.83 (t, $J = 6.8$ Hz, 2H), 3.77 – 3.22 (m, 4H), 1.83 – 1.65 (m, 3H), 1.65 – 1.42 (br. m, 3H). ^{13}C and APT NMR (101 MHz, CDCl_3) δ 171.28 (C=O), 140.79 (C), 140.68 (C), 127.75 (C), 126.53 (CH), 125.35 (CH), 123.07 (C), 122.88 (C), 120.78 (CH), 120.11 (CH), 120.01 (CH), 108.89 (CH), 108.38 (CH), 44.90 (CH_2), 41.28 (CH_2), 26.28 (CH_2), 24.82 (CH_2). ESI: m/z 341.1 (M + H) $^+$. HRMS calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{OCl}$ (M + H) $^+$ 341.1421, found 341.1382.

4.1.57. (9-(3-Chloropropyl)-9H-carbazol-3-yl)(piperidin-1-yl)methanone (57)—

Using carboxylic acid **53** (800 mg, 2.78 mmol) and piperidine (604 μ L, 6.11 mmol) as starting compounds, the title compound was prepared as a colorless glass according to the procedure described above for compound **4**. Yield: 540 mg (55%). ^1H NMR (500 MHz, CDCl_3) δ 8.19 (d, $J = 1.1$ Hz, 1H), 8.09 (d, $J = 7.8$ Hz, 1H), 7.54 (dd, $J = 8.4, 1.6$ Hz, 1H), 7.52 – 7.43 (m, 3H), 7.26 (ddd, $J = 7.9, 6.1, 2.0$ Hz, 1H), 4.51 (t, $J = 6.5$ Hz, 2H), 3.94 – 3.30 (m, 4H), 3.50 (t, $J = 6$ Hz, 2H), 2.42 – 2.26 (m, 2H), 1.76 – 1.67 (br. m, 3H), 1.63 (br. s, 3H). ^{13}C and APT NMR (126 MHz, CDCl_3) δ 171.46 (C=O), 141.01 (C), 140.90 (C), 127.34 (C), 126.49 (CH), 125.35 (CH), 122.98 (C), 122.72 (C), 120.75 (CH), 120.02 (CH), 119.80 (CH), 109.00 (CH), 108.41 (CH), 42.34 (CH_2), 39.99 (CH_2), 31.84 (CH_2), 24.88 (CH_2). ESI: m/z 355.1 (M + H) $^+$. HRMS calcd for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{OCl}$ (M + H) $^+$ 355.1577, found 355.1579.

4.1.58. (9-(2-Iodoethyl)-9H-carbazol-3-yl)(piperidin-1-yl)methanone (58)—

Powdered NaI (5.78 g, 38.56 mmole) was added to a solution of compound **56** (1.95 g, 5.71 mmole) in acetonitrile (100 mL) under nitrogen. After stirring at reflux for 72 h, the reaction mixture was filtered, and the solvent was removed under reduced pressure. The obtained residue was extracted with EtOAc (150 mL). The extract was washed consecutively with 5% aqueous sodium dithionite (50 mL, 2 times), brine, dried over MgSO_4 , and filtered. The solvent was evaporated under reduced pressure, and the residue self-crystallized. The white crystalline solid was collected by filtration, washed with heptanes (20 mL) and dried *in vacuo*. Yield: 2.22 g (90%); mp 161°C. According to the NMR and mass-spec analyses the final product represented a 4:1 mixture of iodide and the unreacted chloride **56**. ^1H NMR (400 MHz, CDCl_3) δ 8.18 (s, 1H), 8.08 (d, $J = 7.8$ Hz, 1H), 7.54 (d, $J = 8.4$ Hz, 1H), 7.49 (t, $J = 7.7$ Hz, 1H), 7.42 (dd, $J = 12.8, 6.3$ Hz, 2H), 7.28 (t, $J = 7.8$ Hz, 1H), 4.69 (t, $J = 7.9$ Hz, 2H), 3.62 (br. s, 4H), 3.43 (t, $J = 7.9$ Hz, 2H), 1.70 (br. m, 3H), 1.63 (br. s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 171.31 (C=O), 140.34 (C), 140.29 (C), 127.89 (C), 126.62 (CH),

125.43 (CH), 123.15 (C), 122.97 (C), 120.92 (CH), 120.25 (CH), 120.16 (CH), 108.85 (CH), 108.31 (CH), 45.91 (CH₂), 26.38 (CH₂), 24.88 (CH₂), -0.06 (CH₂).

4.1.59. (9-(3-Iodopropyl)-9H-carbazol-3-yl)(piperidin-1-yl)methanone (59)—

Powdered NaI (3.7 g, 24.68 mmole) was added to a solution of compound **57** (1.247 g, 3.51 mmole) in acetonitrile (100 mL) under nitrogen. After stirring at reflux for 72 h, the reaction mixture was filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography on silica gel using EtOAc/heptane in different proportions. The resulting colorless oil was dried in high *vacuo* to yield the target compound as a colorless glass. Yield: 1.328 g (85%). ¹H NMR (500 MHz, CDCl₃) δ 8.09 (s, 1H), 7.98 (d, *J* = 7.7 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 1H), 7.41 – 7.31 (m, 3H), 7.16 (t, *J* = 6.5 Hz, 1H), 4.29 (t, *J* = 6.5 Hz, 2H), 3.92 – 3.17 (br. m, 4H), 3.02 (t, *J* = 6.4 Hz, 2H), 2.36 – 2.17 (m, 2H), 1.59 (br. s, 3H), 1.53 (br. s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 171.33 (C=O), 140.85 (C), 140.76 (C), 127.17 (C), 126.39 (CH), 125.23 (CH), 122.85 (C), 122.62 (C), 120.66 (CH), 119.94 (CH), 119.71 (CH), 109.03 (CH), 108.46 (CH), 43.22 (CH₂), 32.71 (CH₂), 24.77 (CH₂), 3.06 (CH₂).

4.1.60. Piperidin-1-yl(9-(2-(piperidin-1-yl)ethyl)-9H-carbazol-3-yl)methanone (60)—

Under nitrogen atmosphere, a solution of compound **58** (600 mg, 1.39 mmol), piperidine (411 μL, 4.17 mmol), and Cs₂CO₃ (904 mg, 2.77 mmol) in acetone (5 mL) was subjected to microwave irradiation at 80°C for 1 h. The reaction mixture was cooled, diluted with ethyl acetate (50 mL), and filtered. The organic solvents were evaporated *in vacuo*, and the obtained residue was dissolved in DCM (150 mL). The organic layer was washed with 1 N NaOH (50 mL × 2), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The crude product was purified on a Biotage® KP-NH cartridge using EtOAc/heptane in different proportions to give the title compound as a pale yellow glass. Yield: 203 mg (37%). ¹H NMR (400 MHz, CDCl₃) δ 8.05 (s, 1H), 7.90 (d, *J* = 7.7 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.33 – 7.19 (m, 3H), 7.06 (t, *J* = 7.3 Hz, 1H), 4.20 (t, *J* = 7.4 Hz, 2H), 3.76 – 3.13 (m, 4H), 2.49 (t, *J* = 7.4 Hz, 2H), 2.28 (br. s, 4H), 1.60 – 1.31 (m, 10H), 1.30 – 1.19 (m, 2H). ¹³C and APT NMR (101 MHz, CDCl₃) δ 171.07 (C=O), 140.62 (C), 140.53 (C), 126.64 (C), 125.87 (CH), 124.78 (CH), 122.48 (C), 122.22 (C), 120.18 (CH), 119.58 (CH), 119.12 (CH), 108.65 (CH), 108.05 (CH), 56.61 (CH₂), 54.74 (CH₂), 40.93 (CH₂), 25.91 (CH₂), 25.74 (CH₂), 24.45 (CH₂), 23.98 (CH₂). ESI: *m/z* 390.2 (M + H)⁺. HRMS calcd for C₂₅H₃₂N₃O (M + H)⁺ 390.2545, found 390.2591.

4.1.61. Piperidin-1-yl(9-(3-(piperidin-1-yl)propyl)-9H-carbazol-3-yl)methanone (61)—

Using iodide **59** (263 mg, 0.59 mmol), piperidine (166 μL, 1.68 mmol), and Cs₂CO₃ (365 mg, 1.12 mmol) in acetone (5 mL) as starting compounds, the title compound was prepared as a light-orange gum according to the procedure described above for compound **60**. Yield: 202 mg (85%). ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.46 (d, *J* = 8.6 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.21 (t, *J* = 6.9 Hz, 1H), 4.34 (t, *J* = 6.5 Hz, 2H), 3.60 (br. s, 4H), 2.26 (br. s, 4H), 2.19 (t, *J* = 6.7 Hz, 2H), 1.98 (p, *J* = 6.4 Hz, 2H), 1.65 (br. s, 3H), 1.63 – 1.51 (m, 7H), 1.41 (br. s, 2H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 171.42 (C=O), 141.03 (C), 140.89 (C), 126.66 (C), 125.95 (CH), 124.82 (CH), 122.64 (C), 122.33 (C), 120.30 (CH), 119.66 (CH), 119.17 (CH), 109.15 (CH), 108.56 (CH), 55.49 (CH₂), 54.35 (CH₂), 40.59 (CH₂), 26.00 (CH₂), 25.92 (CH₂), 24.66 (CH₂), 24.44 (CH₂). ESI: *m/z* 404.1 (M + H)⁺. HRMS calcd for C₂₆H₃₄N₃O (M + H)⁺ 404.2702, found 404.2675.

4.1.62. Piperidin-1-yl(9-(3-(1,1-dioxo-thiomorpholino))-9H-carbazol-3-yl)methanone (62)—

Using iodide **59** (290 mg, 0.65 mmol), 1,1-dioxo-thiomorpholine (227 mg, 1.68 mmol), and Cs₂CO₃ (365 mg, 1.12 mmol) in acetone (5 mL) as starting

compounds, the title compound was prepared as a colorless gum according to the procedure described above for compound **60**. Yield: 112 mg (38%). ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 8.07 (d, *J* = 7.7, 1H), 7.51 (d, *J* = 8.3, 1H), 7.48 – 7.34 (m, 3H), 7.23 (t, *J* = 7.1, 1H), 4.34 (t, *J* = 6.6 Hz, 2H), 3.61 (br. s, 4H), 2.83 (br. s, 4H), 2.73 (br. s, 4H), 2.32 (t, *J* = 6.6 Hz, 2H), 2.02 – 1.90 (m, 2H), 1.68 (br. s, 3H), 1.62 (br. s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.05 (C=O), 140.71 (C), 126.89 (C), 126.01 (CH), 124.87 (CH), 122.51 (C), 122.25 (C), 120.41 (CH), 119.66 (CH), 119.37 (CH), 108.90 (CH), 108.28 (CH), 53.31 (CH₂), 50.89 (CH₂), 50.46 (CH₂), 40.24 (CH₂), 25.51 (CH₂), 24.53 (CH₂). ESI: *m/z* 454.2 (M + H)⁺. HRMS calcd for C₂₅H₃₂N₃O₃S (M + H)⁺ 454.2164, found 454.2132.

4.1.63. 3-(3-(Piperidine-1-carbonyl)-9*H*-carbazol-9-yl)propyl (1,1-dioxo-thiomorpholino)-4-carboxylate (63)—The title compound was obtained as a by-product in the form of a light yellow glass from the synthesis of compound **62**. Yield: 75 mg (23%). ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.54 (d, *J* = 8.3 Hz, 1H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.37 (t, *J* = 8.5 Hz, 2H), 7.27 (dd, *J* = 8.6, 6.5 Hz, 1H), 4.43 (t, *J* = 6.1 Hz, 2H), 4.18 (t, *J* = 5.6 Hz, 2H), 3.86 (br. s, 2H), 3.77 – 3.25 (br. m, 6H), 2.92 (br. s, 2H), 2.76 (br. s, 2H), 2.41 – 2.17 (m, 2H), 1.70 (br. s, 3H), 1.64 (br. s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.23 (C=O), 154.26 (C=O), 140.75 (C), 127.45 (C), 126.46 (CH), 125.38 (CH), 122.88 (C), 122.59 (C), 120.81 (CH), 119.96 (CH), 119.84 (CH), 108.82 (CH), 108.23 (CH), 64.56 (CH₂), 51.75 (CH₂), 42.52 (CH₂), 40.54 (CH₂), 28.28 (CH₂), 24.81 (CH₂). ESI: *m/z* 498.2 (M + H)⁺. HRMS calcd for C₂₆H₃₂N₃O₅S (M + H)⁺ 498.2063, found 498.2085.

4.1.64. *N*-(3-(3-(Piperidine-1-carbonyl)-9*H*-carbazol-9-yl)propyl)methanesulfonamide (64)—Sodium hydride (60% dispersion in mineral oil, 108 mg, 2.82 mmol) was added portionwise to a solution of compound **59** (247 mg, 0.55 mmol) in dry DMF (3 mL) under nitrogen atmosphere, followed by methanesulfonamide (257 mg, 2.70 mmol) at 0°C. The reaction mixture was stirred for 16 h while warming at room temperature. After quenching with water, the reaction mixture was extracted with EtOAc (150 mL), washed with water (5×50 mL), then brine, dried (MgSO₄), filtered and concentrated *in vacuo*. The crude product was purified using EtOAc/heptane in different proportions to give the title compound as a colorless gum. Yield: 206 mg (90.6%). ¹H NMR (400 MHz, CDCl₃) δ 8.15 (s, 1H), 8.02 (d, *J* = 7.7, 1H), 7.38 (t, *J* = 7.5, 1H), 7.30 (d, *J* = 8.5, 2H), 7.19 (t, *J* = 6.8, 2H), 6.11 (s, 1H), 4.16 (t, *J* = 6.7, 2H), 3.68 (br. s, 2H), 3.45 (br. s, 2H), 2.98 (t, *J* = 7.1 Hz, 2H), 2.79 (s, 3H), 1.97 – 1.84 (m, 2H), 1.66 (br. s, 3H), 1.58 (br. s, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 171.53 (C=O), 140.62 (C), 140.55 (C), 126.61 (C), 126.38 (CH), 124.84 (CH), 122.53 (CH), 122.45 (C), 120.49 (C), 119.72 (CH), 119.58 (CH), 108.97 (CH), 108.36 (CH), 40.77 (CH₂), 40.13 (CH₂), 39.49 (CH₃), 29.01 (CH₂), 24.60 (CH₂). ESI: *m/z* 414.1 (M + H)⁺. HRMS calcd for C₂₂H₂₈N₃O₃S (M + H)⁺ 414.1851, found 414.1853.

4.1.65. Methyl 4-(phenylamino)benzoate (65)—Under argon atmosphere, a solution of methyl 4-bromobenzoate (3.5 g, 16.28 mmol), aniline (1.819 g, 19.53 mmol), palladium (II) acetate (218 mg, 0.97 mmol), *rac*-BINAP (506 mg, 0.81 mmol), and potassium carbonate (6.72 g, 48.62 mmol) in toluene (ca. 10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 160°C for 2 h and then cooled to rt. The reaction mixture was diluted with DCM and filtered. The organic solvents were evaporated *in vacuo*, and the residue was suspended in methyl *tert*-butyl ether (150 mL). The organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography (gradient elution starting with 5% EtOAc in heptane to 60% EtOAc in heptane) to afford title compound **65** (3.55 g, 96% yield) as a pale green solid: mp

121°C. ¹H NMR (500 MHz, CDCl₃) δ 7.94 – 7.87 (m, 2H), 7.35 – 7.29 (m, 2H), 7.16 (d, *J* = 7.5 Hz, 2H), 7.05 (t, *J* = 7.4 Hz, 1H), 7.00 – 6.95 (m, 2H), 6.15 (s, 1H), 3.86 (s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 167.19 (C=O), 148.27 (C), 140.99 (C), 131.63 (CH), 129.64 (CH), 123.21 (CH), 121.10 (C), 120.55 (CH), 114.69 (CH), 51.91 (CH₃).

4.1.66. Methyl 9*H*-carbazole-3-carboxylate (66)—In a 100 mL round-bottom flask, a mixture of palladium acetate (1.821 g, 8.11 mmol) and diphenylamine **36** (1.676 g, 7.37 mmol) in glacial acetic acid (40 mL) was stirred under reflux for 1 hr. The organic solvent was removed by distillation. The precipitated metallic palladium was separated by transferring the obtained black residue into a folded paper filter and continuous extraction with acetone in a Soxhlet extractor until the condensing solvent turned colorless. The extract was concentrated *in vacuo*, and the resultant solid was sonicated for 10 min in a bath sonicator with 1 M hydrochloric acid (100 mL), filtered, rinsed with distilled water (50 mL × 3), and then dried *in vacuo*. The dry precipitate was sublimed under vacuum to afford the title compound as a light yellow solid. Yield: 1.081 g (65%); mp 180°C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.73 (s, 1H), 8.81 (d, *J* = 1.6 Hz, 1H), 8.25 (d, *J* = 7.8 Hz, 1H), 8.03 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.56 (t, *J* = 8.3 Hz, 2H), 7.45 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H), 7.25 – 7.20 (m, 1H), 3.89 (s, 3H). ¹H NMR (500 MHz, CDCl₃) δ 8.84 – 8.79 (m, 1H), 8.50 (s, 1H), 8.12 (dd, *J* = 8.5, 1.7 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.38 (d, *J* = 8.6 Hz, 1H), 7.27 (ddd, *J* = 8.0, 6.6, 1.5 Hz, 1H), 3.97 (s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 168.24 (C=O), 142.52 (C), 140.14 (C), 127.59 (CH), 126.74 (CH), 123.43 (C), 123.26 (C), 123.07 (CH), 121.39 (C), 120.78 (CH), 120.47 (CH), 111.13 (CH), 110.38 (CH), 52.21 (CH₃).

4.1.67. (9*H*-carbazol-3-yl)(piperidin-1-yl)methanone (67)—Potassium hydroxide (3 g, 53.47 mmol) was added to a stirred solution of methyl ester **37** (818 mg, 3.63 mmol) in a mixture of ethanol (40 mL) and water (10 mL). The reaction mixture was stirred at reflux for 16 hrs and then cooled to room temperature. The solvents were evaporated under reduced pressure, and the residue was diluted with DI water. Using external cooling (ice-bath), the solution was acidified to pH *ca.* 2 by dropwise addition of 1M aqueous HCl. The precipitated product was extracted with EtOAc (150 mL). The organic layer was washed with brine under acidic pH, dried over MgSO₄, and filtered. The solvent was then evaporated under reduced pressure, and the resulting residue was purified by silica chromatography using 70% EtOAc in heptane to yield the title compound as a beige solid. Yield: 737 mg (96%); mp 271°C. The obtained 9*H*-carbazole-3-carboxylic acid (742 mg, 3.51 mmol), piperidine (416 μL, 4.21 mmol), DIPEA (966 μL, 5.68 mmol), and DMAP (43 mg, 0.35 mmol) were added to DCM (30 mL) under nitrogen. The obtained solution was cooled down on an ice-water bath. EDAC (808 mg, 4.21 mmol) was added to the solution, and the reaction mixture was stirred for 16 h while warming at room temperature. The solvent was removed *in vacuo*, and the obtained residue was extracted with EtOAc (150 mL). The organic layer was washed consecutively with 5% citric acid solution (50 mL × 3), concentrated sodium bicarbonate (50 mL × 3), and brine (50 mL). The organic phase was then dried (MgSO₄), filtered, and concentrated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with EtOAc/heptanes in different proportions to give the title compound as a beige solid. Yield: 839 mg (86%); mp 221°C. ¹H NMR (500 MHz, DMSO-*d*₆) δ 11.47 (s, 1H), 8.18 (d, *J* = 8.0 Hz, 2H), 7.51 (dd, *J* = 8.2, 1.8 Hz, 2H), 7.42 (dd, *J* = 4.4, 1.3 Hz, 1H), 7.41 – 7.38 (m, 1H), 7.18 (t, *J* = 7.4 Hz, 1H), 3.50 (s, 4H), 1.67 – 1.57 (m, 2H), 1.52 (s, 4H). ¹³C NMR and DEPT (126 MHz, DMSO-*d*₆) δ 170.10 (C=O), 140.16 (C), 140.08 (C), 126.56 (C), 126.00 (CH), 124.69 (CH), 122.31 (C), 121.91 (C), 120.53 (CH), 119.50 (CH), 118.92 (CH), 111.16 (CH), 110.48 (CH), 25.72 (CH₂), 24.19 (CH₂). ESI: *m/z* 279.1 (M + H)⁺. HRMS calcd for C₁₈H₁₉N₂O (M + H)⁺ 279.1497, found 279.1514.

4.1.68. 9-(3-Methoxypropyl)-9H-carbazol-3-yl(piperidin-1-yl)methanone (68)—

Under argon atmosphere, a solution of the carbazole amide **67** (100 mg, 0.36 mmol), 1-bromo-3-methoxypropane (61 μ L, 0.54 mmol), and Cs_2CO_3 (234 mg, 0.72 mmol) in DMF (10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 140°C for 1 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc and filtered. The organic solvents were evaporated *in vacuo*. The residue was suspended in methyl *tert*-butyl ether (150 mL), and the organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO_4), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel eluting with EtOAc/heptanes in different proportions to give the title product (119 mg, 95%) as a clear, colorless gum. ^1H NMR (500 MHz, CDCl_3) δ 8.19 (s, 1H), 8.08 (d, $J = 7.8$ Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 1H), 7.50 – 7.40 (m, 3H), 7.24 (ddd, $J = 8.0, 6.6, 1.3$ Hz, 1H), 4.41 (t, $J = 6.5$ Hz, 2H), 3.64 (s, 4H), 3.29 (s, 3H), 3.23 (t, $J = 5.6$ Hz, 2H), 2.08 (p, $J = 6.04$ Hz, 2H), 1.73 – 1.66 (m, 2H), 1.62 (s, 4H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 171.48 (C=O), 141.09 (C), 140.95 (C), 126.87 (C), 126.19 (CH), 125.08 (CH), 122.73 (C), 122.43 (C), 120.48 (CH), 119.81 (CH), 119.38 (CH), 109.01 (CH), 108.39 (CH), 68.91 (CH_2), 58.60 (CH_3), 39.57 (CH_2), 29.10 (CH_2), 24.76 (CH_2). ESI: m/z 351.1 (M + H) $^+$. HRMS calcd for $\text{C}_{22}\text{H}_{27}\text{N}_2\text{O}_2$ (M + H) $^+$ 351.2073, found 351.2048.

4.1.69. Piperidin-1-yl(9-((tetrahydro-2H-pyran-4-yl)methyl)-9H-carbazol-3-yl)methanone (69)—

Using carbazole amide **67** (100 mg, 0.36 mmol) and 4-(bromomethyl)tetrahydro-2H-pyran (97 mg, 0.54 mmol) as starting compounds, the title compound was prepared as a colorless glass according to the procedure described above for compound **68**. Yield: 120 mg (89%). ^1H NMR (500 MHz, CDCl_3) δ 8.18 (d, $J = 1.2$ Hz, 1H), 8.08 (d, $J = 7.7$ Hz, 1H), 7.53 (dd, $J = 8.4, 1.6$ Hz, 1H), 7.47 (ddd, $J = 8.3, 7.1, 1.3$ Hz, 1H), 7.38 (t, $J = 8.7$ Hz, 2H), 7.27 – 7.21 (m, 1H), 4.15 (d, $J = 7.3$ Hz, 2H), 3.93 (t, $J = 2.8$ Hz, 1H), 3.91 (t, $J = 2.9$ Hz, 1H), 3.49 (br. m, 4H), 3.30 – 3.21 (m, 2H), 2.23 (dp, $J = 15.1, 7.4$ Hz, 1H), 1.68 (br. s, 2H), 1.62 (br. s, 4H), 1.51 – 1.45 (m, 4H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 171.35 (C=O), 141.21 (C), 141.14 (C), 126.99 (C), 126.20 (CH), 125.12 (CH), 122.66 (C), 122.38 (C), 120.54 (CH), 119.80 (CH), 119.47 (CH), 109.18 (CH), 108.64 (CH), 67.49 (CH_2), 48.98 (CH_2), 35.60 (CH), 31.18 (CH_2), 24.75 (CH_2). ESI: m/z 377.1 (M + H) $^+$. HRMS calcd for $\text{C}_{24}\text{H}_{29}\text{N}_2\text{O}_2$ (M + H) $^+$ 377.2229, found 377.2192.

4.1.70. Methyl 4-(3-(piperidine-1-carbonyl)-9H-carbazol-9-yl)butanoate (70)—

Using carbazole amide **67** (100 mg, 0.36 mmol) and methyl 4-bromobutanoate (54 μ L, 0.43 mmol) as starting compounds, the title compound was prepared as a colorless glass according to the procedure described above for compound **68**. Yield: 127 mg (93%). ^1H NMR (500 MHz, CDCl_3) δ 8.19 (s, 1H), 8.07 (d, $J = 7.7$, 1H), 7.52 (d, $J = 8.4$, 1H), 7.49 – 7.44 (m, 1H), 7.40 (t, $J = 8.0$, 2H), 7.24 (t, $J = 7.4$, 1H), 4.36 (t, $J = 7.0$, 2H), 3.64 (s, 3H), 3.60 (br.s, 4H), 2.31 (t, $J = 7.0$, 2H), 2.21 – 2.14 (m, 2H), 1.68 (br.s, 3H), 1.62 (br.s, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 173.28 (C=O), 171.38 (C=O), 140.83 (C), 140.74 (C), 127.01 (C), 126.26 (CH), 125.12 (CH), 122.76 (C), 122.51 (C), 120.56 (CH), 119.88 (CH), 119.50 (CH), 108.92 (CH), 108.30 (CH), 51.72 (CH_3), 41.96 (CH_2), 30.75 (CH_2), 24.74 (CH_2), 23.87 (CH_2). ESI: m/z 379.1 (M + H) $^+$. HRMS calcd for $\text{C}_{23}\text{H}_{27}\text{N}_2\text{O}_3$ (M + H) $^+$ 379.2022, found 379.1993.

4.1.71. (9-(3-(Dimethylamino)propyl)-9H-carbazol-3-yl)(piperidin-1-yl)methanone (71)—

Under argon atmosphere, a solution of carbazole amide **67** (248 mg, 0.89 mmol), 3-chloropropyl dimethylamine hydrochloride (311 mg, 1.97 mmol), TBAI (128 mg, 0.35 mmol), and Cs_2CO_3 (886 mg, 2.72 mmol) in DMF (10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 140°C for 2 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc (50

mL) and filtered. The organic solvents were evaporated *in vacuo*. The residue was suspended in methyl *tert*-butyl ether (150 mL), and the organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on a Biotage® KP-NH (amino-modified silica gel) cartridge using heptanes/EtOAc in different proportions to afford the title compound as a light yellow viscous gum. Yield: 180 mg, 56%. ¹H NMR (500 MHz, CDCl₃) δ 8.19 (d, *J* = 1.5 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.53 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.48 – 7.42 (m, 3H), 7.23 (ddd, *J* = 8.0, 4.7, 3.4 Hz, 1H), 4.36 (t, *J* = 6.8 Hz, 2H), 3.62 (br.s, 4H), 2.22 (t, *J* = 6.7 Hz, 2H), 2.19 (s, 6H), 1.98 (p, *J* = 6.7 Hz, 2H), 1.67 (br.s, 3H), 1.61 (br.s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.47 (C=O), 141.02 (C), 140.90 (C), 126.74 (C), 126.11 (CH), 125.00 (CH), 122.68 (C), 122.39 (C), 120.42 (CH), 119.75 (CH), 119.27 (CH), 109.08 (CH), 108.49 (CH), 56.38 (CH₂), 45.42 (CH₃), 40.66 (CH₂), 26.89 (CH₂), 24.72 (CH₂). ESI: *m/z* 364.1 (M + H)⁺. HRMS calcd for C₂₃H₃₀N₃O (M + H)⁺ 364.2389, found 364.2359.

4.1.72. *Tert*-butyl 3-(3-(piperidine-1-carbonyl)-9*H*-carbazol-9-yl)propylcarbamate (72)—

Using amide **67** (435 mg, 1.56 mmol) and *tert*-butyl *N*-(3-bromopropyl)-carbamate (558 mg, 2.34 mmol) as starting compounds, the title compound was prepared as a light yellow glass according to the procedure described above for compound **71**. Yield: 617 mg (91 %). ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 8.08 (d, *J* = 7.7 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.46 (d, *J* = 7.5 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 8.6 Hz, 1H), 7.28 – 7.21 (m, 1H), 4.68 (br. s, 1H), 4.34 (t, *J* = 6.4 Hz, 2H), 3.63 (br. s, 4H), 3.22 – 3.04 (m, 2H), 2.14 – 1.98 (m, 2H), 1.70 (br. s, 3H), 1.63 (br. s, 3H), 1.43 (s, 9H). ¹³C and DEPT NMR (101 MHz, CDCl₃) δ 171.54 (C=O), 156.20 (C=O), 140.88 (C), 140.82 (C), 127.17 (C), 126.43 (CH), 125.29 (CH), 122.96 (C), 122.71 (C), 120.77 (CH), 120.06 (CH), 119.66 (CH), 108.94 (CH), 108.31 (CH), 79.59 (C), 40.75 (CH₂), 38.60 (CH₂), 29.36 (CH₂), 28.55 (CH₃), 24.90 (CH₂). ESI: *m/z* 436.3 (M + H)⁺. HRMS calcd for C₂₆H₃₄N₃O₃ (M + H)⁺ 436.2600, found 436.2644.

4.1.73. 9-(3-Aminopropyl)-9*H*-carbazol-3-yl(piperidin-1-yl)methanone (73)—

Starting with compound **72** (354 mg, 0.81 mmol), the title compound was prepared as a light yellow glass according to the analogous procedure described above for compound **29**. The obtained residue was purified by flash chromatography eluting with EtOAc/EtOH (gradient elution) on a Biotage® KP-NH cartridge to give 230 mg (84 %) of the target product in the form of a light amber glass. ¹H NMR (500 MHz, CDCl₃) δ 8.15 (d, *J* = 1.5 Hz, 1H), 8.04 (d, *J* = 7.8 Hz, 1H), 7.47 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.44 – 7.39 (m, 1H), 7.39 – 7.34 (m, 2H), 7.21 – 7.17 (m, 1H), 4.30 (t, *J* = 6.8 Hz, 2H), 3.82 – 3.32 (br. m, 4H), 2.61 (t, *J* = 6.8 Hz, 2H), 1.90 (p, *J* = 6.8 Hz, 2H), 1.70 – 1.60 (m, 3H), 1.57 (br. s, 3H), 1.47 (s, 2H). ¹³C and APT NMR (126 MHz, CDCl₃) δ 171.30 (C=O), 140.81 (C), 140.71 (C), 126.67 (C), 126.05 (CH), 124.90 (CH), 122.54 (C), 122.27 (C), 120.36 (CH), 119.64 (CH), 119.23 (CH), 108.91 (CH), 108.32 (CH), 49.00 (CH₂), 43.46 (CH₂), 40.39 (CH₂), 39.27 (CH₂), 32.16 (CH₂), 26.02 (CH₂), 24.59 (CH₂). ESI: *m/z* 336.2 (M + H)⁺. HRMS calcd for C₂₁H₂₆N₃O (M + H)⁺ 336.2076, found 336.2069.

4.1.74. Piperidin-1-yl(9-(pyridin-4-ylmethyl)-9*H*-carbazol-3-yl)methanone (74)—

Amide **67** (100 mg, 0.36 mmol) and potassium *tert*-butoxide (305 mg, 2.71 mmol) were added to DMF (5 mL) in a 25 mL round bottom flask. 4-(Bromomethyl)pyridine hydrobromide (136 mg, 0.54 mmol) was added to the reaction mixture in one portion. The reaction mixture was stirred at room temperature for 2 days, diluted with EtOAc (25 mL) and filtered. The solvents were evaporated *in vacuo*, and the obtained syrup was extracted with EtOAc. The organic layer was washed with an aqueous sodium bicarbonate, brine, dried (MgSO₄) and filtered. The volatiles were removed *in vacuo*, and the obtained syrup

was purified on a Biotage® KP-NH cartridge eluting with EtOAc/heptanes in different proportions to afford the target product as a light yellow glass. Yield: 118 mg (89%). ¹H NMR (500 MHz, CDCl₃) δ 8.48 (d, *J* = 5.0 Hz, 2H), 8.23 (s, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.29 (t, *J* = 8.0 Hz, 2H), 7.27 – 7.23 (m, 1H), 6.97 (d, *J* = 5.4 Hz, 2H), 5.48 (s, 2H), 3.58 (br.m, 4H), 1.79 – 1.67 (m, 3H), 1.63 (br.s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.22 (C=O), 150.35 (CH), 145.93 (C), 140.90 (C), 140.86 (C), 127.88 (C), 126.69 (CH), 125.43 (CH), 123.04 (C), 122.91 (C), 121.40 (CH), 120.85 (CH), 120.26 (CH), 120.07 (CH), 108.96 (CH), 108.41 (CH), 45.66 (CH₂), 24.78 (CH₂). ESI: *m/z* 370.2 (M + H)⁺. HRMS calcd for C₂₄H₂₄N₃O (M + H)⁺ 370.1919, found 370.1886.

4.1.75. Piperidin-1-yl(9-(pyridin-3-ylmethyl)-9H-carbazol-3-yl)methanone (75)—

Using amide **67** (100 mg, 0.36 mmol) potassium *tert*-butoxide (160 mg, 1.42 mmol) and 3-(bromomethyl)pyridine hydrobromide (136 mg, 0.54 mmol) as starting compounds, the title compound was prepared as a colorless glass according to the procedure described above for compound **26**. Yield: 45 mg (34%). ¹H NMR (500 MHz, CDCl₃) δ 8.56 (s, 1H), 8.48 (s, 1H), 8.21 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.51 – 7.47 (m, 1H), 7.47 – 7.41 (m, 1H), 7.37 – 7.30 (m, 2H), 7.30 – 7.23 (m, 2H), 7.15 – 7.09 (m, 1H), 5.49 (d, *J* = 4.1 Hz, 2H), 3.55 (br.m, 4H), 1.68 (br.s, 3H), 1.61 (br.s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.27 (C=O), 149.31 (CH), 148.36 (CH), 140.89 (C), 140.85 (C), 134.23 (CH), 132.53 (C), 127.79 (C), 126.67 (CH), 125.43 (CH), 123.85 (CH), 123.10 (C), 122.95 (C), 120.84 (CH), 120.17 (CH), 120.07 (CH), 109.03 (CH), 108.48 (CH), 44.34 (CH₂), 24.82 (CH₂). ESI: *m/z* 370.1 (M + H)⁺. HRMS calcd for C₂₄H₂₄N₃O (M + H)⁺ 370.1919, found 370.1895.

4.1.76. Piperidin-1-yl(9-(pyridin-2-ylmethyl)-9H-carbazol-3-yl)methanone (76)—

Using carbazole amide **67** (100 mg, 0.36 mmol) and potassium *tert*-butoxide (305 mg, 2.72 mmol) were added to DMF (5 mL) in a 25 mL round bottom flask. 2-(Bromomethyl)pyridine hydrobromide (340 mg, 1.34 mmol) was added to the reaction mixture in one portion. The reaction mixture was stirred at room temperature for 2 days, diluted with EtOAc (25 mL) and filtered. The solvents were evaporated *in vacuo*, and the obtained syrup was extracted with EtOAc. The organic layer was washed with an aqueous sodium bicarbonate, brine, dried (MgSO₄) and filtered. The volatiles were removed *in vacuo*, and the obtained syrup was purified on a Biotage® KP-NH cartridge eluting with EtOAc/heptanes in different proportions to afford the target product as a light yellow glass. Yield 106 mg (80%). ¹H NMR (500 MHz, CDCl₃) δ 8.61 (ddd, *J* = 4.9, 1.9, 1.0 Hz, 1H), 8.23 (d, *J* = 1.1 Hz, 1H), 8.12 (d, *J* = 7.7 Hz, 1H), 7.50 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.47 – 7.42 (m, 1H), 7.41 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.39 – 7.34 (m, 2H), 7.29 – 7.25 (m, 1H), 7.13 (dd, *J* = 7.1, 5.3 Hz, 1H), 6.62 (d, *J* = 7.9 Hz, 1H), 5.62 (s, 2H), 3.69 (br.m, 4H), 1.78 – 1.66 (m, 3H), 1.62 (br.s, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.31 (C=O), 156.79 (C), 149.63 (CH), 141.05(C), 140.99 (CH), 137.15 (CH), 127.60 (C), 126.54 (CH), 125.33 (CH), 123.01 (C), 122.85 (C), 122.64 (CH), 120.69 (C), 120.48 (CH), 120.00 (CH), 119.95 (CH), 109.19 (CH), 108.67 (CH), 48.69 (CH₂), 24.77 (CH₂). ESI: *m/z* 370.2 (M + H)⁺. HRMS calcd for C₂₄H₂₄N₃O (M + H)⁺ 370.1919, found 370.1901.

4.1.77. Methyl 4-(3-methoxyphenylamino)benzoate (77)—

Under argon atmosphere, a solution of methyl 4-bromobenzoate (3.5 g, 16.28 mmol), 3-methoxyaniline (2 g, 16.24 mmol), palladium (II) acetate (218 mg, 0.97 mmol), *rac*-BINAP (506 mg, 0.81 mmol), and potassium carbonate (6.72 g, 48.62 mmol) in toluene (*ca.* 10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 160°C for 2 h. The reaction mixture was allowed to cool down to room temperature, diluted with DCM (50 mL) and filtered. The solvents were removed under reduced pressure, and the obtained residue was distilled *in vacuo* to afford a light yellow oil: b.p. 160–165°C at 0.2 mm Hg.

Yield: 3.348 g (80%). ^1H NMR (500 MHz, CDCl_3) δ 7.93 – 7.88 (m, 2H), 7.22 (t, J = 8.1 Hz, 1H), 7.02 – 6.98 (m, 2H), 6.75 (ddd, J = 8.0, 2.1, 0.9 Hz, 1H), 6.72 (t, J = 2.2 Hz, 1H), 6.60 (ddd, J = 8.3, 2.4, 0.7 Hz, 1H), 6.18 (s, 1H), 3.87 (s, 3H), 3.78 (s, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 167.17 (C=O), 160.78 (C), 147.99 (C), 142.36 (C), 131.60 (CH), 130.39 (CH), 121.27 (C), 115.09 (CH), 112.68 (CH), 108.35 (CH), 106.07 (CH), 55.42 (CH_3), 51.92 (CH_3).

4.1.78. Methyl 7-methoxy-9H-carbazole-3-carboxylate (Clausine C or clauszoline-L, 78)—Using benzoate **77** (1.897 g, 7.37 mmol) and palladium acetate (1.987 g, 8.85 mmol) as starting compounds, the title compound was prepared following the procedures described in preparation of compound **66** as a pale yellow solid. Yield: 1.6 g (85%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 11.57 (s, 1H), 8.66 (d, J = 1.6 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 7.93 (dd, J = 8.5, 1.7 Hz, 1H), 7.49 (dd, J = 8.5, 0.5 Hz, 1H), 7.02 (d, J = 2.2 Hz, 1H), 6.83 (dd, J = 8.6, 2.3 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H). ^{13}C NMR and DEPT (126 MHz, $\text{DMSO}-d_6$) δ 167.06 (C=O), 159.06 (C), 142.67 (C), 141.83 (C), 125.53 (CH), 122.55 (C), 121.48 (CH), 121.19 (CH), 119.89 (C), 116.05 (C), 110.45 (CH), 108.75 (CH), 94.86 (CH), 55.32 (CH_3), 51.73 (CH_3).

4.1.79. Methyl 7-methoxy-9-pentyl-9H-carbazole-3-carboxylate (79)—Under argon atmosphere, a solution of carbazole **78** (462 mg, 1.81 mmol), 1-bromopentane (320 μL , 2.59 mmol), and Cs_2CO_3 (1.123 g, 3.45 mmol) in DMF (10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 140°C for 2 h and then cooled to rt. The reaction mixture was diluted with EtOAc and filtered. The organic solvents were evaporated *in vacuo*. The residue was suspended in methyl *tert*-butyl ether (150 mL), and the organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO_4), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on silica gel, eluent: EtOAc–heptanes (1/99, v/v) \rightarrow EtOAc–heptanes (2/3, v/v) to give the title compound (480 mg, 82%) as a light yellow viscous oil. ^1H NMR (500 MHz, CDCl_3) δ 8.70 (d, J = 1.2 Hz, 1H), 8.08 (dd, J = 8.6, 1.7 Hz, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.31 (d, J = 8.6 Hz, 1H), 6.88 (dd, J = 8.5, 2.2 Hz, 1H), 6.85 (d, J = 2.1 Hz, 1H), 4.20 (t, J = 7.3 Hz, 2H), 3.96 (s, 3H), 3.92 (s, 3H), 1.84 (p, J = 7.3 Hz, 2H), 1.38 – 1.30 (m, 4H), 0.87 (t, J = 7.0 Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 168.17 (C=O), 159.61 (C), 143.48 (C), 142.56 (C), 126.25 (CH), 122.92 (C), 122.01 (CH), 121.54 (CH), 120.84 (C), 116.95 (C), 108.06 (CH), 108.04 (CH), 93.88 (CH), 55.88 (CH_3), 52.06 (CH_3), 43.37 (CH_2), 29.50 (CH_2), 28.64 (CH_2), 22.62 (CH_2), 14.12 (CH_3).

4.1.80. 7-Methoxy-9-pentyl-9H-carbazole-3-carboxylic acid (80)—Potassium hydroxide (3 g, 53.47 mmol) was added to a stirred solution of methyl ester **79** (626 mg, 1.92 mmol) in a mixture of ethanol (40 mL) and water (10 mL). The reaction mixture was stirred at reflux for 16 h and then cooled to room temperature. The solvents were evaporated under reduced pressure, and the residue was diluted with DI water. The solution was placed in an ice-water bath, and acidified to pH *ca.* 2 by dropwise addition of 1M aqueous HCl. The precipitated product was extracted with EtOAc, washed with brine under acidic pH (*ca.* 2), and dried over MgSO_4 . After evaporation of the solvent under reduced pressure, the residue was chromatographed on silica gel with 70% EtOAc in heptanes to yield the title compound as a beige solid. Yield: 480 mg (80%); mp 171°C. ^1H NMR (600 MHz, CDCl_3) δ 8.79 (d, J = 1.3 Hz, 1H), 8.18 (dd, J = 8.6, 1.6 Hz, 1H), 8.01 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 8.6 Hz, 1H), 6.91 (dd, J = 8.5, 2.1 Hz, 1H), 6.87 (d, J = 2.0 Hz, 1H), 4.22 (t, J = 7.3 Hz, 2H), 3.94 (s, 3H), 1.86 (p, J = 7.21 Hz, 2H), 1.42 – 1.31 (m, 4H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C NMR and DEPT (126 MHz, CDCl_3) δ 173.33 (C=O), 159.70 (C), 144.05 (C), 142.65 (C), 126.95 (CH), 123.06 (C), 122.86 (CH), 121.68 (CH), 119.94 (C), 116.98 (C), 108.22 (CH),

108.19 (CH), 94.01 (CH), 55.95 (CH₃), 43.47 (CH₂), 29.54 (CH₂), 28.68 (CH₂), 22.66 (CH₂), 14.16 (CH₃).

4.1.81. (7-Methoxy-9-pentyl-9H-carbazol-3-yl)(piperidin-1-yl)methanone (81)—

Carboxylic acid **80** (1 g, 3.21 mmol), piperidine (636 μ L, 6.42 mmol), DIPEA (1.1 mL, 6.42 mmol), and DMAP (785 mg, 6.42 mmol) were added to DCM (100 mL) under nitrogen. The obtained solution was cooled down on an ice-water bath. EDC (1231 mg, 6.42 mmol) was added to the solution, and the reaction mixture was stirred for 16 h while warming at room temperature. The solvent was removed *in vacuo*, and the obtained residue was extracted with EtOAc (150 mL). The organic layer was washed consecutively with 5% citric acid solution (50 mL \times 3), concentrated sodium bicarbonate (50 mL \times 3), brine (50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified on silica gel using heptanes/EtOAc (gradient elution) to afford the title compound as a light yellow gum that self-solidified upon standing in the refrigerator to a light yellow solid. Yield: 1109 mg, (91%); mp 96°C. ¹H NMR (500 MHz, CDCl₃) δ 8.07 (d, *J* = 1.3 Hz, 1H), 7.94 (d, 1H), 7.45 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.32 (d, *J* = 8.4 Hz, 1H), 6.86 (dq, *J* = 4.3, 2.2 Hz, 2H), 4.22 (t, *J* = 7.2 Hz, 2H), 3.93 (s, 3H), 3.63 (br.s, 4H), 1.85 (p, *J* = 7.3 Hz, 2H), 1.74 – 1.66 (m, 3H), 1.62 (br. s, 3H), 1.38 – 1.31 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H). ¹³C NMR and DEPT (126 MHz, CDCl₃) δ 171.73 (C=O), 159.45 (C), 142.37 (C), 141.25 (C), 126.97 (C), 123.86 (CH), 122.75 (C), 121.38 (CH), 119.03 (CH), 116.73 (C), 108.18 (CH), 107.62 (CH), 93.57 (CH), 55.84 (CH₃), 43.26 (CH₂), 29.48 (CH₂), 28.64 (CH₂), 26.37 (CH₂), 24.88 (CH₂), 22.62 (CH₂), 14.11 (CH₃). ESI: *m/z* 379.3 (M + H)⁺. HRMS calcd for C₂₄H₃₁N₂O₂ (M + H)⁺ 379.2386, found 379.2390.

4.1.82. Ethyl 3-(3-(4-methylpiperazine-1-carbonyl)-9H-carbazol-9-yl)propanoate (82)—

Using carboxylic acid **51** (240 mg, 0.77 mmol) and 1-methylpiperazine (103 μ L, 0.93 mmol) as starting compounds, the title compound was prepared as a light amber gum according to the procedure described above for compound **4** with the exception of not using 5% citric acid solution during the workup. Yield: 268 mg (88%). ¹H NMR (500 MHz, CDCl₃) δ 8.19 (d, *J* = 1.3 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.54 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.50 – 7.37 (m, 3H), 7.23 (ddd, *J* = 7.9, 6.8, 1.3 Hz, 1H), 4.59 (t, *J* = 7.0 Hz, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 3.71 (br. s, 4H), 2.81 (t, *J* = 7.0 Hz, 2H), 2.44 (br. s, 4H), 2.31 (s, 3H), 1.11 (t, *J* = 7.2 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 171.17 (C=O), 171.08 (C=O), 140.51 (C), 140.31 (C), 126.33 (C), 126.26 (CH), 125.18 (CH), 122.71 (C), 122.60 (C), 120.45 (CH), 120.01 (CH), 119.64 (CH), 108.89 (CH), 108.37 (CH), 60.80 (CH₂), 45.98 (CH₃), 38.71 (CH₂), 33.39 (CH₂), 13.92 (CH₃). ESI: *m/z* 394.3 (M + H)⁺. HRMS calcd for C₂₃H₂₈N₃O₃ (M + H)⁺ 393.2131, found 394.2091.

4.1.83. Ethyl 3-(3-((1,1-dioxo-thiomorpholino)-4-carbonyl)-9H-carbazol-9-yl)propanoate (83)—

Using 9-(3-ethoxy-3-oxopropyl)-9H-carbazole-3-carboxylic acid (400 mg, 1.28 mmol) and 1,1-dioxo-thiomorpholine (209 mg, 1.55 mmol) as starting compounds, the title compound was prepared as a colorless glass following the procedures described in preparation of compound **4**. Yield: 289 mg, (52%). ¹H NMR (500 MHz, CDCl₃) δ 8.14 (d, *J* = 1.0 Hz, 1H), 8.00 (d, *J* = 7.8 Hz, 1H), 7.48 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.46 – 7.39 (m, 3H), 7.21 (ddd, *J* = 8.0, 6.8, 1.2 Hz, 1H), 4.58 (t, *J* = 7.0 Hz, 2H), 4.09 (br. s, 4H), 4.00 (q, *J* = 7.2 Hz, 2H), 3.02 (br. s, 4H), 2.77 (t, *J* = 7.0 Hz, 2H), 1.07 (t, *J* = 7.2 Hz, 3H). ¹³C and DEPT NMR (126 MHz, CDCl₃) δ 172.35 (C=O), 171.28 (C=O), 141.32 (C), 140.66 (C), 126.94 (CH), 125.21 (CH), 124.42 (C), 123.13 (C), 122.73 (C), 120.80 (CH), 120.51 (CH), 120.29 (CH), 109.29 (CH), 109.03 (CH), 61.20 (CH₂), 52.18 (CH₂), 39.04 (CH₂), 33.63 (CH₂), 14.17 (CH₃). ESI: *m/z* 429.3 (M + H)⁺. HRMS calcd for C₂₂H₂₅N₂O₅S (M + H)⁺ 429.1484, found 429.1476.

4.1.84. (9*H*-carbazol-3-yl)(1,1-dioxothiomorpholino)methanone (84)—Using 9*H*-carbazole-3-carboxylic acid (204 mg, 0.97 mmol), thiomorpholine 1,1-dioxide (154 mg, 1.14 mmol), DIPEA (330 μ L, 1.89 mmol), DMAP (12 mg, 0.1 mmol), and EDAC (218 mg, 1.14 mmol), the title compound was prepared as an off-white solid following the procedures described in preparation of compound **67**. Yield: 119 mg (38%); mp 326°C. ^1H NMR (500 MHz, DMSO- d_6) δ 11.54 (s, 1H), 8.34 (s, 1H), 8.19 (d, $J = 7.8$ Hz, 1H), 7.59 – 7.50 (m, 3H), 7.43 (t, $J = 7.5$ Hz, 1H), 7.20 (t, $J = 7.4$ Hz, 1H), 3.96 (br. s, 4H), 3.31 (br. s, 4H). ^{13}C and DEPT NMR (126 MHz, DMSO- d_6) δ 170.79 (C=O), 140.46 (C), 140.21 (C), 126.17 (CH), 125.02 (CH), 124.88 (C), 122.28 (C), 121.91 (C), 120.57 (CH), 119.93 (CH), 119.08 (CH), 111.26 (CH), 110.70 (CH), 50.97 (CH₂).

4.1.85. *Tert*-butyl 3-(3-((1,1-dioxo-thiomorpholino)-1-carbonyl)-9*H*-carbazol-9-yl)propyl-carbamate (85)—Under argon atmosphere, a solution of carbazole amide **84** (114 mg, 0.35 mmol), *tert*-butyl *N*-(3-bromopropyl)-carbamate (161 mg, 0.68 mmol), and powdered NaOH (21 mg, 0.53 mmol) in acetone (10 mL) was tightly capped in a 20 mL microwave vessel. The mixture was subjected to microwave irradiation at 80°C for 1 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc (50 mL) and filtered. The organic solvents were evaporated *in vacuo*. The residue was suspended in methyl *tert*-butyl ether (150 mL), and the organic phase was washed with saturated aqueous sodium bicarbonate, brine, dried (MgSO₄), filtered and evaporated *in vacuo*. The obtained residue was purified by column chromatography on a Biotage® KP-NH (amino-modified silica gel) cartridge using heptanes/EtOAc in different proportions to afford the title compound as a colorless glass. Yield: 143 mg (84%). ^1H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1H), 8.06 (d, $J = 7.7$ Hz, 1H), 7.49 (t, $J = 10.0$ Hz, 2H), 7.43 – 7.33 (m, 2H), 7.30 – 7.21 (m, 1H), 4.83 (s, 1H), 4.32 (t, $J = 6.8$ Hz, 2H), 4.14 (br. s, 4H), 3.21 – 2.98 (m, 6H), 2.03 (p, $J = 6.2$ Hz, 2H), 1.43 (s, 9H). ^{13}C and APT NMR (101 MHz, CDCl₃) δ 172.26 (C=O), 156.09 (C=O), 141.38 (C), 140.79 (C), 126.78 (CH), 125.11 (CH), 124.13 (C), 122.81 (C), 122.48 (C), 120.71 (CH), 120.41 (CH), 119.95 (CH), 109.07 (CH), 108.68 (CH), 79.47 (C), 52.04 (CH₂), 40.68 (CH₂), 38.45 (CH₂), 29.24 (CH₂), 28.45 (CH₃). ESI: m/z 486.0 (M + H)⁺. HRMS calcd for C₂₅H₃₂N₃O₅S (M + H)⁺ 486.2063, found 486.2076.

4.2. CB2-agonist structural characterization through ligand-steered modeling

The crude model of the active-state CB2 receptor was based on a multi-template approach [5, 35], using the crystal structures of A2A adenosine receptor (A2AR) in the active state bound to agonist UK-432097[36] (PDB 3QAK), and the sphingosine 1-phosphate receptor 1 (S1P1R) bound to an antagonist sphingolipid mimic[37] (PDB 3V2Y). A multiple alignment of CB2, S1P1R, A2AR, together with the sequences of CB1, S1P2R, S1P3R, S1P4R, S1P5RL, lysophosphatidic acid receptor 1 (LPAR1), LPAR2 and LPAR3 was built using BLAST[34] and taking into account the information of the key conserved residues within class-A GPCRs[38]. The extracted alignment of CB2, S1P1R and A2AR is shown in Fig. S1. The two-residue gap in helix 5 of CB2 and S1P1R was introduced according to the structural superposition of GPCR crystal structures[39]. The nomenclature of Ballesteros and Weinstein[40] is used throughout the text, whereby X.50 denotes the most conserved residue in helix X. In agreement with Gonzalez et al.[39], residue L201 was considered as 5.50 in CB2. The alignment was used as input in ICM[41] to build a preliminary model of CB2 using the A2AR in the active state as template (3QAK), with the exception of the E2-loop and helix 5, which taking into account both sequence similarity and the smaller number of gaps, were built using S1P1R (3V2Y) as template. The N-term and C-term CB2 residues (1-29 and 316-360, respectively), and the TL4 residues connecting helices 4 and 5 in the A2AR structure were deleted. The disulfide bond within the E2-loop between C174 and C179 was inherited from the S1P1R template, and is in agreement with the work of others[42, 43]. This crude model was subjected to a restraint energy minimization to relieve

structural strain stemming from the non-conservative substitutions. The refinement of the binding site was performed using the ligand-steered modeling method[6, 44–46]. Starting from the crude model developed through the multitemplate approach described above, agonist **WIN55,212-2** was extracted from the GPCR Ligand Library (GLL)[47] (<http://cavasotto-lab.net/Databases/GDD/>), and seeded into the pocket, and a structural set of 50 structures was generated by randomizing the position and orientation of the ligand. Then, a multistep energy minimization was performed, in which the van der Waals interaction was gradually switched on, as already described[48, 49]. The ligand and receptor were held flexible in this stage without restraints. The structures in the set were subjected to a full flexible ligand-flexible side chain Monte Carlo-based global energy optimization in ICM[50–53]. The top-ranking structures were then visually inspected for ligand-receptor interactions, and a CB2-**WIN55,212-2** complex was thus selected. It is observed that the interaction between the ligand and W5.43 revealed by mutagenesis analysis[54] was present in our model. The CB2 structural model thus obtained was used model complexes between compounds and CB2 using the ligand-steered method outlined above.

4.3. In vitro receptor radioligand binding studies

CB1 and CB2 radioligand binding data were obtained using National Institute of Mental Health (NIMH) Psychoactive Drug Screening Program (PDSP) resources as described earlier[7, 55–57]. Compounds were screened in a competitive binding experiment using, respectively, membrane fractions prepared from rat brain homogenate expressing CB1 receptor and HEK293 cells expressing the human CB2 receptor. The competition binding experiment for CB1 and CB2 was performed in 96 well plates containing Standard Binding Buffer (50 mM Tris HCl, 1 mM EDTA, 3 mM MgCl₂, 5 mg/ml fatty acid-free BSA, pH 7.4). The radioligand was [³H]**CP55,940**, and the reference compound was compound **CP55,940**. A solution of the compound to be tested was prepared as a 1 mg/ml stock in DMSO and then diluted in Standard Binding Buffer by serial dilution. Radioligand was diluted to five times the assay concentration in Standard Binding Buffer. Aliquots (50 μl) of radioligand were dispensed into the wells of a 96-well plate containing 100 μl of Standard Binding Buffer. Then, duplicate 50-μl aliquots of the test and reference compound dilutions were added. Finally, crude membrane fractions of cells were resuspended in 3 ml of chilled Standard Binding Buffer and homogenized by several passages through a 26 gauge needle, then 50 μl are dispensed into each well. The 250-μl reactions were incubated at room temperature for 1.5 hours, and then harvested by rapid filtration onto Whatman GF/B glass fiber filters pre-soaked with 0.3% polyethyleneimine using a 96-well Brandel harvester. Four rapid 500-μl washes were performed. Filters were placed in 6-ml scintillation tubes and allowed to dry overnight. Bound radioactivity was harvested onto 0.3% polyethyleneimine-treated, 96-well filter mats using a 96-well Filtermate harvester. The filter mats were dried, then scintillant was melted onto the filters and the radioactivity retained on the filters counted in a Microbeta scintillation counter. Raw data (dpm) representing total radioligand binding (i.e., specific + non-specific binding) were plotted as a function of the logarithm of the molar concentration of the competitor (i.e., test or reference compound). Non-linear regression of the normalized (i.e., percent radioligand binding compared to that observed in the absence of test or reference compound) raw data was performed in Prism 4.0 (GraphPad Software) using the built-in three parameter logistic model describing ligand competition binding to radioligand-labeled sites: $y = \text{bottom} + [(\text{top}-\text{bottom})/(1 + 10^{\text{x}-\log\text{IC}_{50}})]$ where bottom equals the residual radioligand binding measured in the presence of 10 μM reference compound (i.e., non-specific binding) and top equals the total radioligand binding observed in the absence of competitor. The log IC₅₀ (i.e., the log of the ligand concentration that reduces radioligand binding by 50%) is thus estimated from the data and used to obtain the K_i by applying the Cheng-Prusoff approximation: $K_i = \text{IC}_{50}/(1 + [\text{ligand}]/K_D)$ where

[ligand] equals the assay radioligand concentration and KD equals the affinity constant of the radioligand for the target receptor.

4.4. [^{35}S]GTP- γ -S functional assays

Functional activity was evaluated using GTP- γ -[^{35}S] assay in Chinese hamster ovarian cell membrane extracts expressing recombinant *hCB1* receptors or *hCB2* receptors. The assay relies on the binding of GTP- γ -[^{35}S], a radiolabeled nonhydrolyzable GTP analogue, to the G protein upon binding of an agonist of the G-protein-coupled receptor. In this system, agonists stimulate GTP- γ -[^{35}S] binding whereas antagonists have no effect and inverse agonists decrease GTP- γ -[^{35}S] basal binding. Compounds were solubilized in 100% DMSO at a concentration of 10 mM within 4 h of the first testing session (master solution). A predilution for the dose-response curve was performed in 100% DMSO and then diluted 100-fold in assay buffer at a concentration fourfold higher than the concentration to be tested. Compounds were tested for agonist activity at eight concentrations in duplicate: 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.001 μ M, with compound **CP55,940** (Tocris, 0949) as the reference agonist. For GTP- γ -[^{35}S], membranes (Euroscreen s.a., Gosselies, Belgium) were mixed with GDP diluted in assay buffer to give 30 μ M solution (volume:volume) and incubated for at least 15 min on ice. In parallel, GTP- γ -[^{35}S] (Amersham, SJ1308) was mixed with the beads PVT-WGA (Amersham, RPNQ001) diluted in assay buffer at 50 mg/ml (0.5 mg/10 μ l) (volume:volume) just before starting the reaction. The following reagents were successively added in the wells of an Optiplate (Perkin Elmer): 50 μ l of ligand, 20 μ l of the membranes:GDP mix, 10 μ l of assay buffer for agonist testing, and 20 μ l of the GTP- γ -[^{35}S]:beads mix. The plates were covered with a topseal, shaken on an orbital shaker for 2 min, and then incubated for 1 h at room temperature. Then the plates were centrifuged for 10 min at 2000 rpm, incubated at room temperature for 1 h, and counted for 1 min/well with a PerkinElmer TopCount reader. Assay reproducibility was monitored by the use of a reference compound **CP55,940**. For replicate determinations, the maximum variability tolerated in the test was of $\pm 20\%$ around the average of the replicates. Efficacies (*E_{max}*) for CB1 and CB2 are expressed as a percentage relative to the efficacy of compound **CP55,940**.

4.5. CB1 and CB2 receptor internalization

The ability of the synthesized compounds to orthosterically interact with CB1 or CB2 receptors was assessed by determining if the compounds either caused internalization on their own (i.e., were agonists), prevented internalization by **CP55,940** (i.e., were antagonists), or had no effect (i.e., were not orthosteric ligands). Receptor internalization was performed as described previously using cells stably expressing either human CB1 receptors or human CB2 receptors[58]. Data are presented as percent of internalization induced by 10 nM **CP55,940** by 3 μ M of each test compound. Thus, no internalization would be 0% and internalization equal to **CP55,940** would be 100%. To determine if the compound was acting as an antagonist, cells were exposed to 10 nM of **CP55,940** and 3 μ M of each test compound. The reversal of internalization by 10 nM of compound **CP55,940** by each test compound was calculated with the following formula: reversal = ((internalization in the presence of test compound with 10 nM **CP55,940**) - (internalization by 10 nM **CP55,940**))/(1 - (internalization by 10 nM **CP55,940**))

4.6. In Vivo Evaluation

4.6.1. Animals—Adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) weighing 120–150 g were used in experimental procedures approved by the Animal Care and Use Committee of The University of Texas M. D. Anderson Cancer Center. Animals were housed three per cage on a 12-h light/12-h dark cycle with water and food pellets available *ad libitum*.

4.6.2. Chronic neuropathic pain model—All surgical procedures were performed under 2–3% isoflurane anesthesia in 100% O₂. The spinal nerve ligation was performed as described previously[59]. Briefly, a midline incision was made in the lumbar spinal region. The left L5 and L6 spinal nerves were isolated under a surgical microscope, and both nerves were tightly ligated with 6-0 silk. A prophylactic antibiotic (5 mg/kg of norfloxacin subcutaneously) and a prophylactic analgesic (0.2–0.5 mg/kg of buprenorphine subcutaneously) were administered once daily for 3 days. All of the behavioral tests were conducted three weeks after spinal nerve ligation.

4.6.3. Drug administration—All dosing solutions were prepared within 1 h prior to injection and stored at room temperature until use. Compound **64** and hydroxypropyl- β -cyclodextrins (30%) were mixed in appropriate ratios in PBS under constant magnetic stirring (200 rpm) at room temperature. **64** dosing solution was administered i.p. as a single bolus injection.

4.6.4. Assessment of mechanical withdrawal thresholds—For assessment of pain hypersensitivity (tactile allodynia), rats were placed in a compartment on a mesh floor and allowed to acclimate for at least 30 min before testing. Mechanical sensitivity was assessed by using a series of von Frey filaments with logarithmic incremental stiffness. A series of calibrated von Frey filaments was used (Stoelting, Wood Dale, IL), as previously described[60], and 50% probability withdrawal thresholds were calculated with the up-down method[61]. In brief, filaments were applied one by one to the plantar surface of the hind paw for 6 s. If no withdrawal response was observed, the next stiffer filament was applied; if there was a withdrawal response, the next less stiff filament was applied. Six consecutive responses from the first change in the response were used to calculate the withdrawal threshold (in grams).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

BSA	bovine serum albumin
CB1	cannabinoid receptor 1
CB2	cannabinoid receptor 2
EC₅₀	half maximal effective concentration
EDTA	ethylenediaminetetraacetic acid
GTP	guanosine-5'-triphosphate

hCB1	human CB1
hCB2	human CB2
IC₅₀	median inhibition concentration

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Appendix A. Supplementary data

Supplementary data related to this article can be found at

- Discovery of a CB2 selective compound **64** based on a carbazole scaffold
- **64** internalized CB2 receptors and activity was confirmed using [³⁵S]GTP- γ -S assays
- **64** had inhibitory effects on pain hypersensitivity in a rat model of neuropathic pain

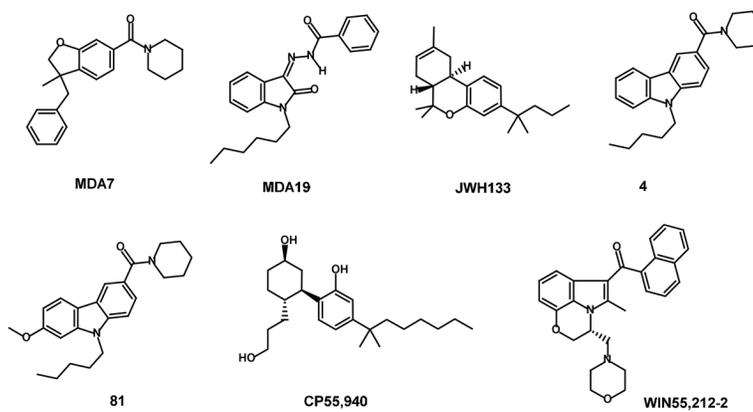


Figure 1. Chemical structures of CB2 modulators (1-7). MDA7[12]; MDA19[17]; JWH133[62]; (4) NMP7[7]; (81) NMP4[7]; CP55,940[63]; WIN55,212-2[64].

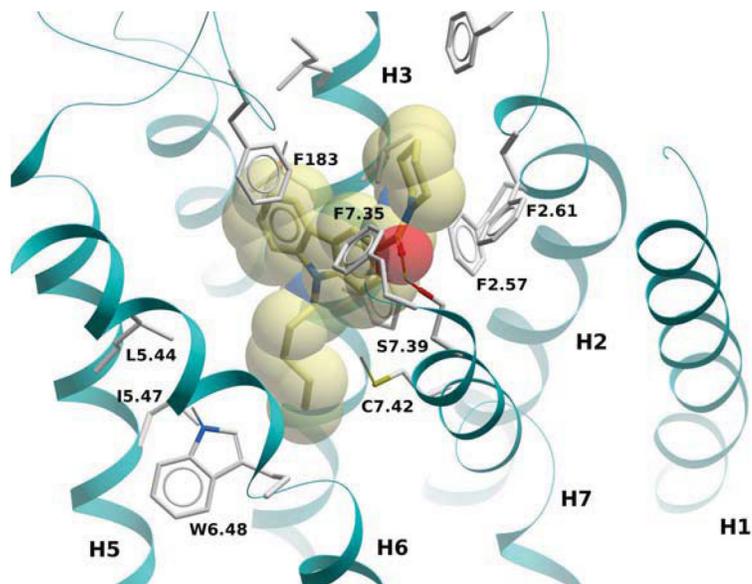


Figure 2. CB2 receptor complexed with compound **4**. The ligand is displayed with yellow carbon atoms, overlaid to a transparent CPK representation. Key receptor residues are displayed as sticks, with light grey carbons. Hydrogen bonds are shown as small spheres.

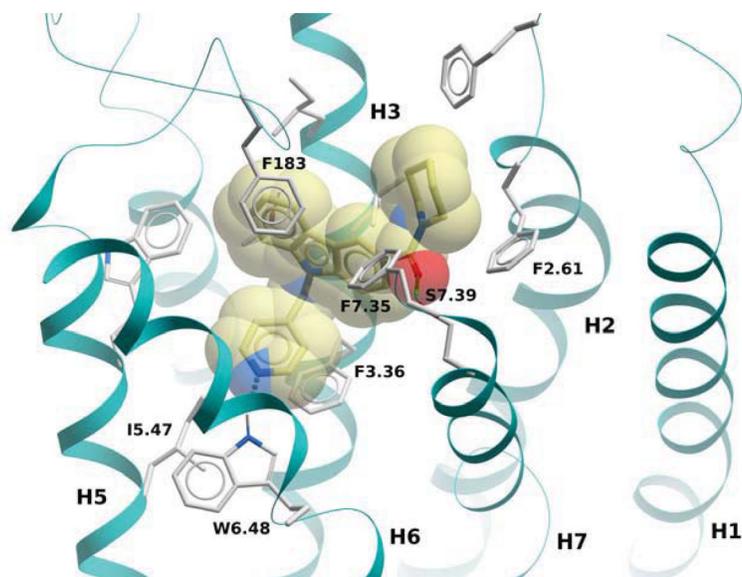


Figure 3. CB2 receptor complexed with compound **74**. The ligand is displayed with yellow carbon atoms, overlaid to a transparent CPK representation. Key receptor residues are displayed as sticks, with light grey carbons. Hydrogen bonds are shown as small spheres.

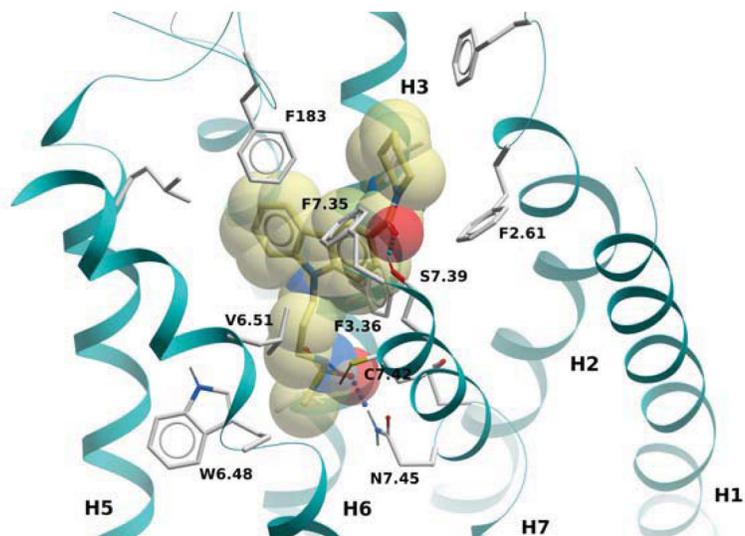


Figure 4. CB2 receptor complexed with compound **64**. The ligand is displayed with yellow carbon atoms, overlaid to a transparent CPK representation. Key receptor residues are displayed as sticks, with light grey carbons. Hydrogen bonds are shown as small spheres.

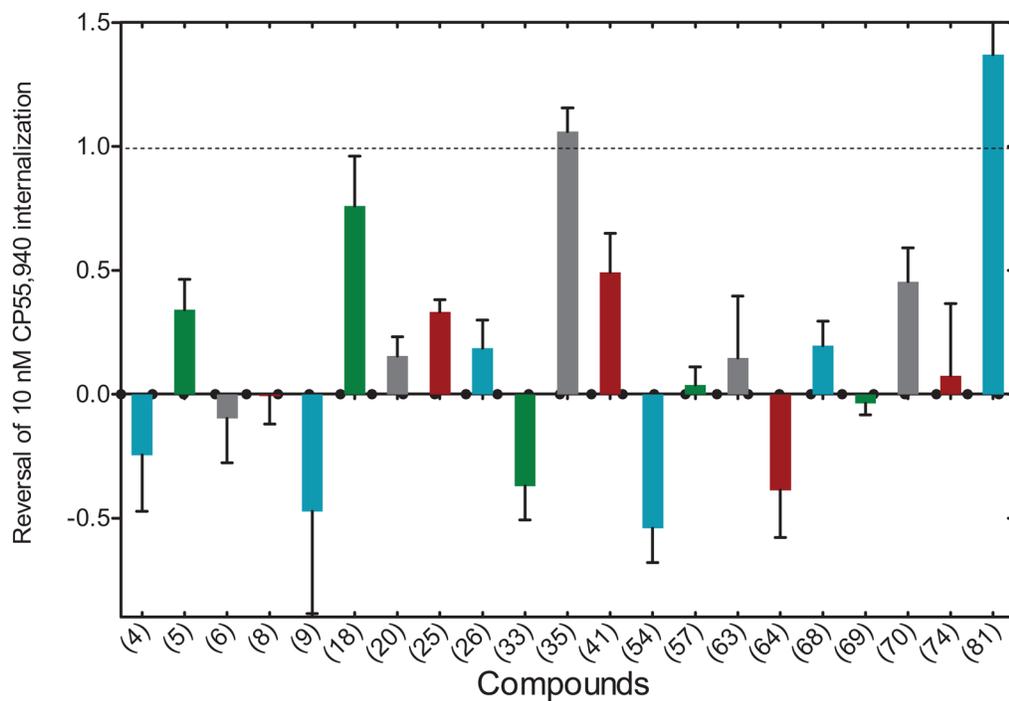


Figure 5. Effects of selected compounds on CP55,940-induced CB2 internalization. hCB2 cells were cotreated with 10 nM of compound CP55,940 and 3 μ M selected compounds.

Compound 64 (n=7)

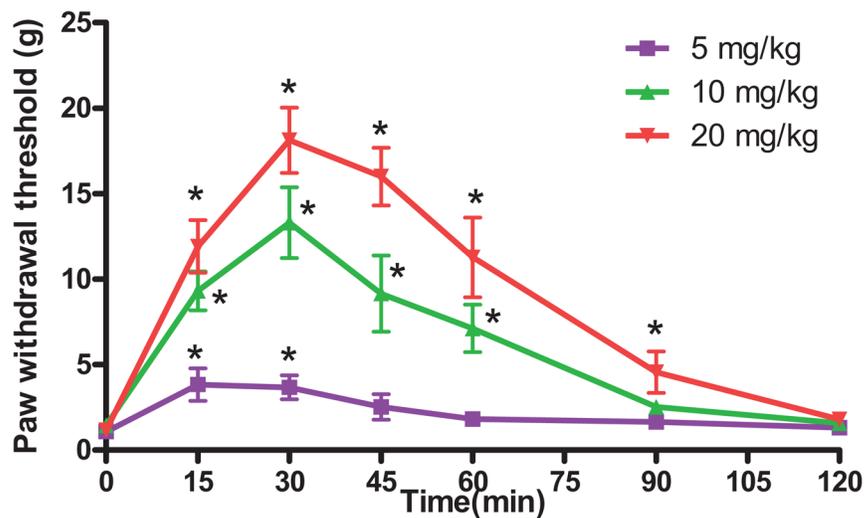
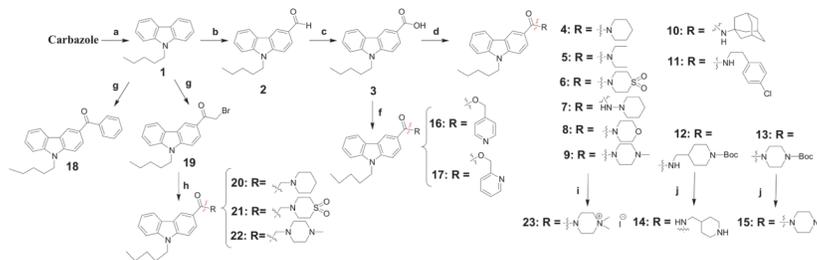
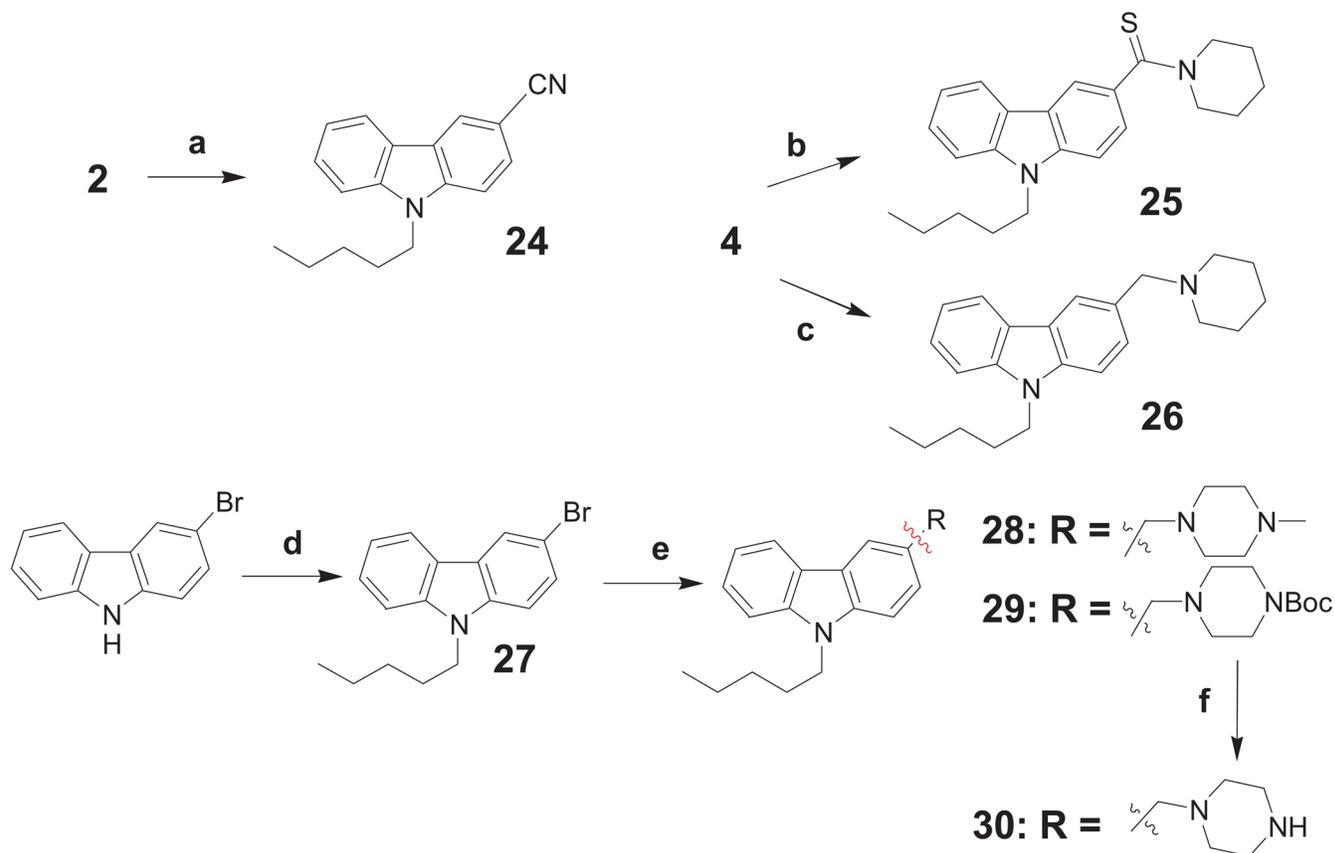


Figure 6. Effect of compound **64** (administered intraperitoneally) on to the paw withdrawal threshold, tested with von Frey filaments, in a neuropathic pain model in rats (seven rats per group). Repeated measures ANOVA with Dunnet's post hoc test were used to determine the statistical difference in each group. * $P < 0.05$ compared with the baseline control (time 0). Data are expressed as mean \pm SEM.

**Scheme 1.**

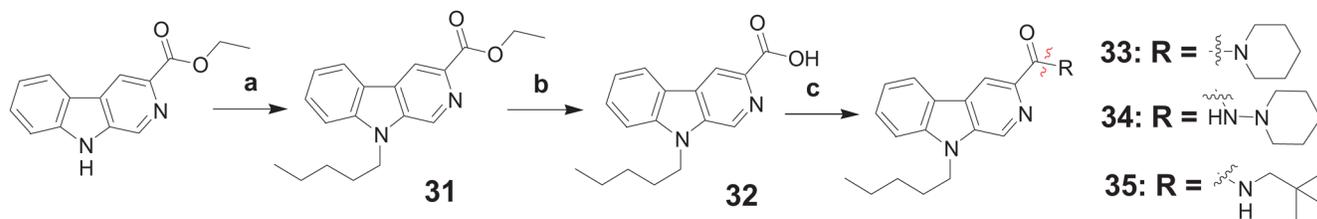
Synthesis of 3-carboxamide 9-pentyl-9H-carbazole derivatives **4-17** and **20-23**.^a

^a Reagents and conditions: (a) *Method A*: *n*-pentyl bromide, Cs₂CO₃, DMF, μ w at 140°C, 1 h. *Method B*: *n*-pentyl bromide, NaOH, acetone, reflux, 16 h; (b) DMF, POCl₃, 0°C → μ w at 100°C, 1h; (c) KMnO₄, acetone-water, 0°C → reflux, 3h; (d) DIEA, DMAP, corresponding amine, EDAC·HCl, DCM, 0°C → rt, 16 h; (f) corresponding bromomethyl pyridines, Et₃N, Na₂CO₃, TBAF, THF, 16 h at rt → μ w at 60°C, 1h; (g) AlCl₃, bromoacetyl bromide or benzoyl chloride, benzene, 0°C → rt, 19 h; (h) corresponding amine, Et₃N, DMF, μ w at 90°C, 5 min; (i) MeI, Et₂O, rt, 18 h; (j) HCl gas, EtOAc, 0°C, 10 min.

**Scheme 2.**

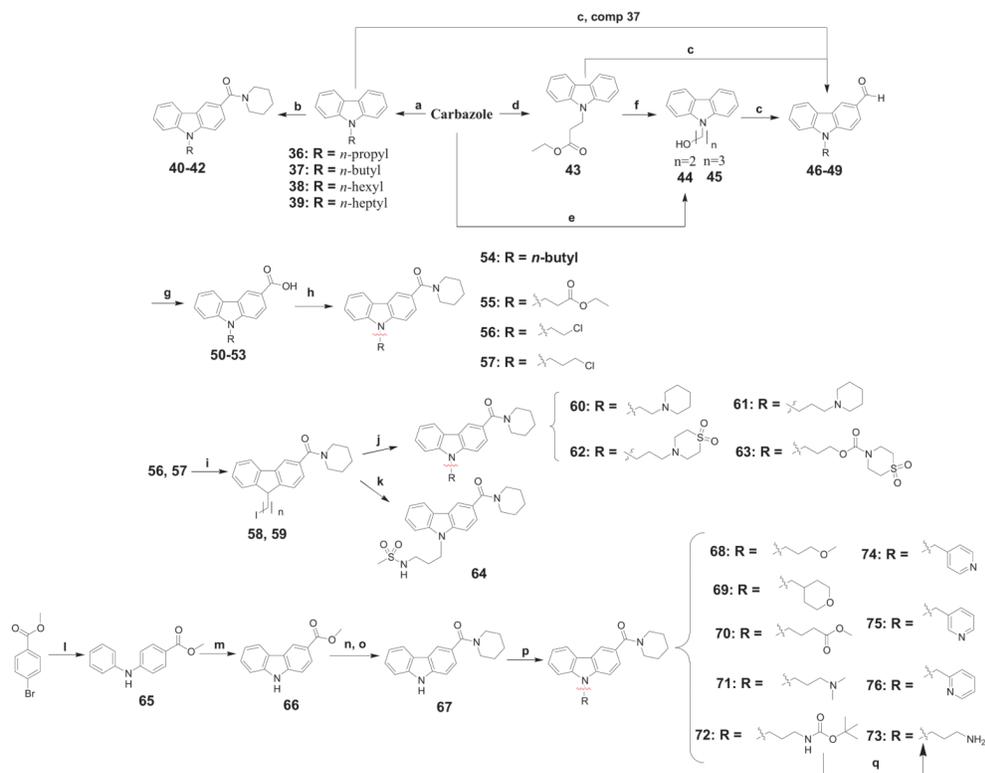
Synthetic modifications of the carbonyl group of 3-carboxamide 9-pentyl-9H-carbazole derivatives **25-26** and **28-30**.^a

^a Reagents and conditions: (a) $\text{NH}_2\text{-OH}\cdot\text{HCl}$, *p*-TSA, $235^\circ\text{C} \rightarrow \mu\text{w}$ at 105°C , 5 min $\rightarrow 235^\circ\text{C}$; (b) LR, toluene, 140°C , 3 h; (c) LAH, THF, $0^\circ\text{C} \rightarrow \text{reflux}$, 10 min; (d) *n*-pentyl bromide, Cs_2CO_3 , DMF, μw at 140°C , 1 h; (e) corresponding potassium BF_3 -methylpiperazinium salts, K_2CO_3 , $\text{Pd}(\text{OAc})_2$, (2-biphenyl)-di-*tert*-butylphosphine, DMF, μw at 120°C , 20 min; (f) TFA, DCM, rt, 6 h.

**Scheme 3.**

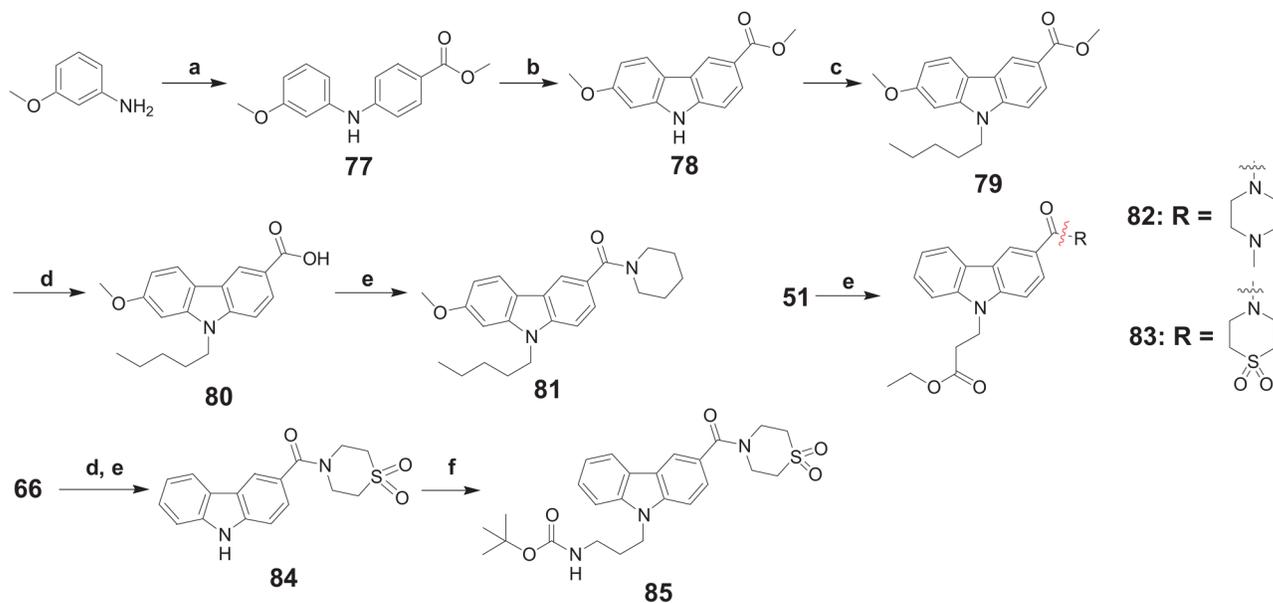
Synthesis of 3-carboxamide 9-pentyl-9*H*-pyrido[3,4-*b*]indole derivatives **33-35**.^a

^a Reagents and conditions: (a) *n*-pentyl bromide, Cs₂CO₃, DMF, μ w at 140°C, 1 h; (b) KOH, ethanol/water, reflux, 16h; (c) DIEA, DMAP, corresponding amine, EDAC·HCl, DCM, 0°C → rt, 16 h.



Scheme 4. Synthesis of (9-substituted-9*H*-carbazol-3-yl)(piperidin-1-yl)methanone derivatives **54-57**, **60-64** and **68-76**.

^a Reagents and conditions: (a) *n*-alkyl bromide or iodide, NaOH, acetone, reflux, 16h; (b) AlCl₃, piperidinecarbonyl chloride, benzene, 0°C → μw at 100°C, 1h; (c) DMF, POCl₃, 0°C → μw at 100°C, 1h; (d) ethyl acrylate, K₂CO₃, DMF, rt, 19h; (e) ethylene oxide, KOH, methyl vinyl ketone, μw at 50°C, 1h; (f) LAH, THF, 0°C → rt, 1h; (g) KMnO₄, acetone-water/acetic acid, 0°C → reflux, 3h; (h) DIEA, DMAP, piperidine, EDAC·HCl, DCM, 0°C → rt, 16h; (i) NaI, acetonitrile, reflux, 72h; (j) corresponding amine, Cs₂CO₃, acetone, rt → 80°C, 1 h; (k) NaH, DMF, 0°C → rt, 16h; (l) aniline, 5 mol% Pd(OAc)₂, 5 mol% rac-BINAP, K₂CO₃, toluene, μW at 160°C, 1h; (m) Pd(OAc)₂, AcOH, reflux, 1 h; (n) KOH, EtOH/H₂O, reflux, 16 h; (o) DIEA, DMAP, piperidine, EDAC·HCl, DCM, 16h; (p) corresponding substituted alkyl bromide, TBAI, Cs₂CO₃, DMF, μW at 140°C, 2 h, or KO*t*-Bu, rt, 16 h; (q) gaseous HCl, EtOAc, 0°C → rt.

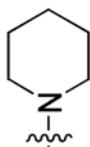
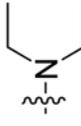
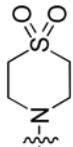
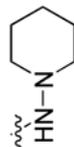
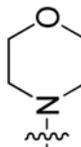
**Scheme 5.**

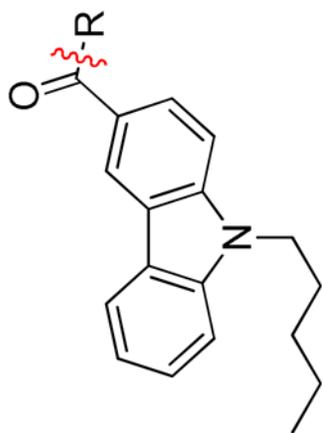
Synthesis of 3-carboxamide-9-substituted-9*H*-carbazol derivatives **81-83** and **85**.^a

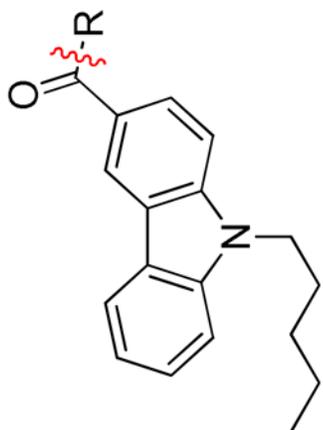
^a Reagents and conditions: (a) methyl 4-bromobenzoate, 5 mol% Pd(OAc)₂, 5 mol% rac-BINAP, K₂CO₃, toluene, μW at 160°C, 2 h; (b) Pd(OAc)₂, AcOH, reflux, 1 h; (c) *n*-pentyl bromide, Cs₂CO₃, DMF, μw at 140°C, 2 h; (d) KOH, EtOH/H₂O, reflux, 16 h; (e) DIEA, DMAP, corresponding amine, EDAC·HCl, DCM, 16 h; (f) *tert*-butyl *N*-(3-bromopropyl)carbamate, NaOH, acetone, μW at 80°C, 1 h.

Table 1

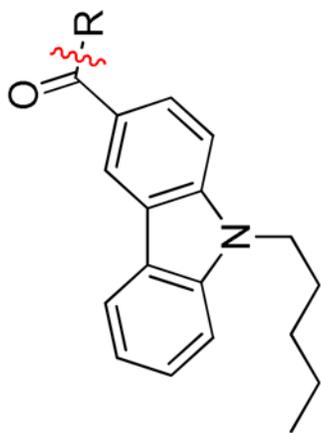
Radioligand competitive binding assays (mean \pm SEM) for 9*H*-carbazole-3-carboxy-based analogues.

Compound	R	TPSA ^a	<i>r</i> CB1 <i>K_i^b</i> (nM)	<i>h</i> CB2 <i>K_i^b</i> (nM)	CB1/CB2 ratio ^c
WIN55,212-2	-	43.70	36.0 \pm 8.3	13.0 \pm 2.1	2.77
4		25.24	77.0 \pm 35.5	73.0 \pm 15.1	1.05
5		25.24	105 \pm 14.6	51.8 \pm 5.6	2.03
6		59.38	440.0 \pm 101.3	225.0 \pm 51.8	1.96
7		37.27	n.b.	1609.0 \pm 279.1	-
8		34.47	60.0 \pm 12.4	84.5 \pm 9.7	0.71





Compound	R	TPSA ^a	rCB1 K _i ^b (nM)	hCB2 K _i ^b (nM)	hCB2 K _i ^b (nM)	CB1/CB2 ratio ^c
9		28.48	123.0 ± 19.8	35.4 ± 4.4	3.47	3.47
10		34.03	1451 ± 273.2	288.5 ± 39.3	5.03	5.03
11		34.03	n.b.	3594.0 ± 424.1	–	–
14		50.64	n.b.	n.b.	–	–
15		41.85	283.0 ± 65.2	2833.0 ± 1304.6	0.10	0.10
16		44.12	108 ± 24.9	1392 ± 320.5	0.08	0.08



Compound	R	TPSA ^a	rCBI K _i ^b (nM)	hCB2 K _i ^b (nM)	hCB2 K _i ^b (nM)	CBI/CB2 ratio ^c
17		44.12	679 ± 156.3	1927 ± 443.7	1927 ± 443.7	0.35
18		22	291.0 ± 67.0	101.0 ± 23.3	101.0 ± 23.3	2.88
20		26.44	3330.0 ± 766.8	1093.0 ± 201.3	1093.0 ± 201.3	3.05
21		59.38	675.0 ± 155.4	502.0 ± 80.9	502.0 ± 80.9	1.34
22		29.68	1504.0 ± 346.3	398.0 ± 64.2	398.0 ± 64.2	3.78
23		25.24	n.b.	n.b.	n.b.	–

^aTopological polar surface area[25].

^bValues are means of three experiments run in triplicates with standard deviation.

K_i of CB1/ K_i of CB2;

n.b. no binding.

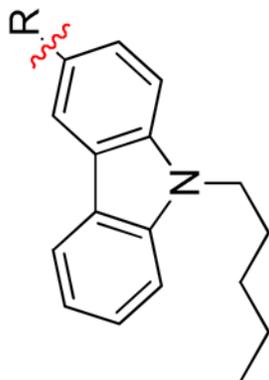
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Table 2

Radioligand competitive binding assays for 9H-carbazole analogues: focusing on the role of the carbonyl group with concomitant variation in basicity.



Compound	R	TPSA ^a	rCB1 K _i ^b (nM)	hCB2 K _i ^b (nM)	CB1/CB2 Ratio ^c
24		28.72	n.b.	n.b.	
25		8.17	40.6 ± 4.2	49.6 ± 4.4	0.82
26		9.37	122.0 ± 28.1	370 ± 85.2	0.33
28		12.61	244.0 ± 56.2	740.0 ± 119.3	0.33
30		24.78	n.b.	n.b.	–

^aTopological polar surface area[25].

^bValues are means of three experiments run in triplicates with standard deviation.

^cK_i of CB1/K_i of CB2;

n.b. no binding.

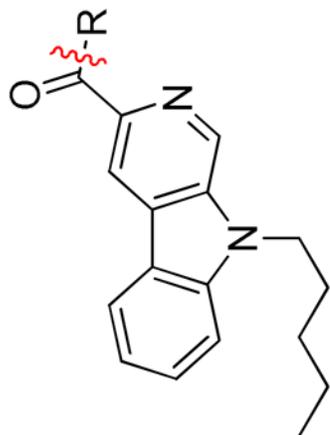
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Table 3

Radioligand competitive binding assays (mean \pm SEM) for the 9*H*-pyrido[3,4-*b*]indole-3-carboxy-based analogues: a tactical approach to assess the impact of potential hydrogen bonding in the 3-carboxy region.



Compound	R	TPSA ^a	rCB1 K _i ^d (nM)	hCB2 K _i ^d (nM)	CB1/CB2 ratio ^b
31		44.12	1859.0 \pm 363.2	1367.0 \pm 194.6	1.36
33		38.13	136.2 \pm 19.9	35.3 \pm 5.5	3.86
34		50.16	354.5 \pm 54.0	171.1 \pm 17.7	2.07
35		46.92	43.6 \pm 6.4	26.5 \pm 4.1	1.65

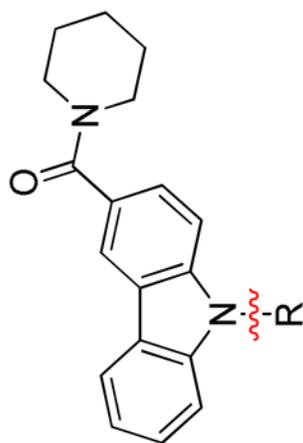
^aTopological polar surface area[25].

^bValues are means of three experiments run in triplicates with standard deviation.

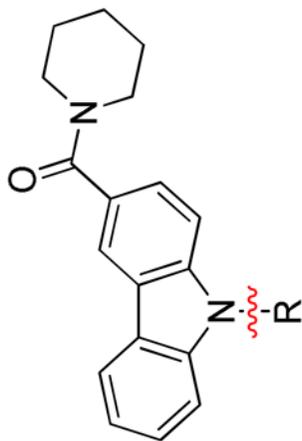
^cK_i of CB1/K_i of CB2.

Radioligand competitive binding assays (mean \pm SEM) for carbazole-based analogues: systematic variation in lipophilicity and basicity in the 9H-region.

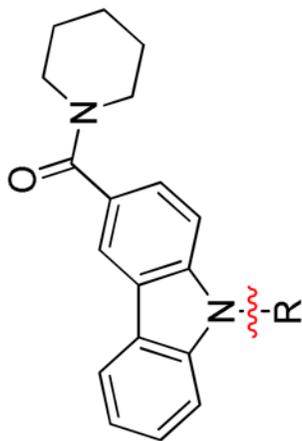
Table 4



Compound	R	TPSA ^a	rCB1 K _i ^b (nM)	hCB2 K _i ^b (nM)	CB1/CB2 Ratio ^c
40		25.24	600.0 \pm 138.2	521.0 \pm 108.0	1.15
41		25.24	241.0 \pm 111.0	96.0 \pm 15.5	2.51
42		25.24	942.5 \pm 433.8	827.0 \pm 171.4	1.14
54		25.24	63.0 \pm 14.5	264 \pm 48.6	0.24
55		51.54	4976 \pm 2291	1313 \pm 242	3.79
56		25.24	2360 \pm 543	519.0 \pm 119	4.55
57		25.24	123.5 \pm 28.3	88.0 \pm 18.2	1.40
60		29.68	n.b.	n.b.	–



Compound	R	TPSA ^a	rCB1 K _i ^b (nM)	hCB2 K _i ^b (nM)	CB1/CB2 Ratio ^c
61		29.68	n.b.	2169 ± 449	–
62		62.62	2376 ± 1094	249.0 ± 40.1	9.54
63		88.92	2256 ± 519	73.0 ± 13.4	30.90
64		71.41	1471 ± 677	62.0 ± 12.8	23.73
67	H	36.10	n.b.	n.b.	–
68		34.47	1442 ± 664	83.0 ± 19.1	8.88
69		34.47	19.5 ± 4.5	20.0 ± 4.6	0.98
70		51.54	1848 ± 425	208.0 ± 47.9	8.88



Compound	R	TPSA ^a	<i>r</i> -CB1 <i>K</i> ₁ ^b (nM)	<i>h</i> -CB2 <i>K</i> ₁ ^b (nM)	CB1/CB2 Ratio ^c
71		29.68	2387 ± 403	403.0 ± 92.8	5.92
72		63.57	n.b.	2690 ± 619	–
73		52.88	n.b.	n.b.	–
74		38.13	393.0 ± 90.5	91.0 ± 21.0	4.32
75		38.13	1024 ± 236	600.0 ± 138.2	1.71
76		38.13	403.0 ± 92.8	215.0 ± 49.5	1.87

^aTopological polar surface area[25].

^bValues are means of three experiments run in triplicates with standard deviation.

^c*K*₁ of CB1/*K*₁ of CB2;

n.b. no binding.

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Table 5

Radioligand competitive binding assays (mean \pm SEM) for carbazole-based analogues with extra and simultaneous modification at two variation points.

Compound	Structure	TPSA ^a	rCB1 K_i^b (nM)	hCB2 K_i^b (nM)	CBI/CB2 Ratio ^c
81		34.47	12.8 \pm 1.8 ^d	7.5 \pm 0.6 ^d	1.71
82		54.78	n.b.	3376 \pm 777	–
83		85.68	n.b.	n.b.	–
85		97.71	n.b.	n.b.	–

^a Topological polar surface area [25].

^b Values are means of three experiments run in triplicates with standard deviation.

^c K_i of CBI/ K_i of CB2;

n.b. no binding.

d_p from ref [7].

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Table 6

Determination of potency (EC_{50}) and maximal stimulation (E_{max}) on *hCB1* and *hCB2* receptors of selected compounds^a

Compound	GTP γ [³⁵ S] functional assays ^a			
	Human CB1		Human CB2	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
CP55,940	N.D.	N.D.	4.13	100
4	96.9 ± 11.9 ^b	73.6 ^b	10.5 ± 1.8 ^b	30.8 ^b
64	N.A.	N.A.	7.3 ± 6.7	59.6
81	118.3 ± 4 ^b	30.4 ^b	9.8 ± 0.3 ^b	-76.4 ^b

^a CB1 and CB2 assay data are presented as the mean of two determinations. Assay reproducibility was monitored by the use of the reference compound CP55,940. For replicate determinations, the maximum variability tolerated in the test was 20% around the average of the replicates. Efficacies (E_{max}) for CB1 or CB2 are expressed as a percentage relative to the efficacy of compound CP55,940. N.D. = not determined; N.A.: not active at 1 μ M;

^b from ref [7].

Table 7Determination of *h*CB1 and *h*CB2 receptor internalization by selected compounds^a.

Compound	Receptor internalization	
	<i>h</i> CB1 (%)	<i>h</i> CB2 (%)
CP55,940	100	100
4	140.4	117.9
5	101.9	53.1
6	23.9	91.5
8	115.3	78.7
9	84.7	43.9
18	77.0	39.3
20	-20.4	102.6
25	124.4	63.0
26	-11.1	79.6
33	127.8	101.1
35	34.7	0.1
41	121.8	77.0
54	84.8	67.9
57	106.3	110.7
63	65.6	27.9
64	54.1	116.2
68	63.2	68.1
69	141.8	86.2
70	9.4	37.3
74	74.9	66.3
81	33.4	-1.1

^a CB1 and CB2 assay data are presented as the mean of two determinations.