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Article

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KEYWORDS

Prostaglandin E2 (PGE2), prostanoid receptors, EP4 subtype, structure activity relationship (SAR), glucuronidation, drug metabolism and pharmacokinetics (DMPK) optimization, endometriosis, anti-nociceptive activity, pain

ABSTRACT

The presence and growth of endometrial tissue outside the uterine cavity in endometriosis patients is primarily driven by hormone-dependent and inflammatory processes - the latter being frequently associated with severe, acute and chronic pelvic pain. The EP4 subtype of prostaglandin E2 (PGE2) receptors (EP4-R) is a particularly promising anti-inflammatory and anti-nociceptive target as both this receptor subtype and the pathways forming PGE2 are highly expressed in endometriotic lesions. High-throughput screening resulted in the identification of benzimidazole derivatives as novel hEP4-R antagonists. Careful SAR investigation guided by rational design identified a methyl substitution adjacent to the carboxylic acid as an appropriate means to accomplish favorable pharmacokinetic properties by reduction of glucuronidation. Further optimization led to the identification of benzimidazolecarboxylic acid **BAY 1316957**, a highly potent, specific and selective hEP4-R antagonist with excellent DMPK properties. Notably, treatment with **BAY 1316957** can be expected to lead to prominent and rapid pain relief and significant improvement of the patients` quality of life.

INTRODUCTION

Endometriosis is one of the most frequent disorders of the female reproductive tract and represents a significant disease burden[1]. It is a chronic condition defined as the presence of endometrial tissue outside the uterine cavity. Viable endometrial cells, most likely derived from retrograde menstruation enter the abdominal cavity, attach to the peritoneum and invade the underlying tissue. As the growth of these cells remains governed by the female hormone cycle their presence is associated with periodic waves of proliferation and subsequent menstruation-like breakdown processes which add substantially to existing inflammatory processes originating from the primary interactions with the previously invaded tissue.[2,3,4] Endometriosis is estimated to affect up to 10% of women of reproductive age in the overall population, however, due to misdiagnosis and diagnostic delay the exact prevalence is not known.[2,3,5] Disease symptoms vary between individual women and can include dysmenorrhea, non-cyclic pelvic pain, dyspareunia, dysuria, dyschesia, fatigue and subfertility.[3,5,6] As a result endometriosis can be both physically and emotionally debilitating, and significantly reduces women's overall quality of life.[1,7,8,9]

Current first- and second-line medical treatments available for endometriosis-related pain include non-steroidal anti-inflammatory drugs (NSAIDs), hormonal therapies, and gonadotropin-releasing hormone (GnRH) agonists and an antagonist. However, these are associated with limitations and unmet needs remain.[1,2,10-14] Guidelines recommend hormonal therapy as empirical treatment to reduce endometriosis-associated pain, however, between 25–30% of patients treated with hormones (including oral contraceptives, vaginal rings, or transdermal patches) do not respond.[2,15] Given that the current treatment options as well as recent approaches in this field[15] primarily address the hormone-dependent aspects of endometriosis, we aimed instead at directly interfering with pro-inflammatory and pro-nociceptive processes resulting

from endometriosis associated inflammation.[17] Notably we wanted to take advantage of the fact, that the pathway leading to the synthesis of PGE2 is highly upregulated in women with endometriosis[18-21] and that both, elevated levels of PGE2 as well as a marked increase in the expression of the EP4-subtype of PGE2 receptors[22] can be found in these patients. The EP4-R has been demonstrated to be responsible for mediating PGE2-induced proliferative and nociceptive effects thus representing a valid target for specific intervention.[21,23] Antagonizing EP4 receptor signaling not only blocked PGE2-induced proliferation of endometrial cells[23,24] but also modulated anti-inflammatory activities[25,26] and attenuated inflammatory pain.[27] As the receptor is also expressed in sensory nociceptive nerve fibres and pain stimulation leads to an increase in expression in dorsal root ganglion (DRG) neurons,[28] antagonism of EP4-receptor signaling can be expected to attenuate inflammatory pain and the subsequent induction of hyperalgesia and allodynia.[29,30] Therefore, antagonism of EP4-R signaling presents a particularly promising approach for treatment of endometriosis-associated inflammatory pain.

The involvement of EP4 receptors in a variety of pathologies and disease states such as migraine, osteoarthritis, rheumatoid arthritis, solid tumors and inflammatory pain is well documented.[26] Consequently the development of EP4-R antagonists has been, and still is, a major focus of medicinal chemistry efforts across the pharmaceutical industry. Either aromatic carboxylic acids or acylsulfonamides are typical structural features common to many of the reported antagonists.[31] The first EP4 receptor antagonist to reach the market was grapiprant (also known as Raqualia-07, RQ-7, AAT-007 or CJ-023,423) which was launched under the trade name Galliprant® by Elanco Animal Health/Aratana for the treatment of osteoarthritis in dogs.[32,33,34] This compound, bearing a distinctive sulfonylurea moiety, was originally discovered by Pfizer/RaQualia.[35] It is also under investigation in humans as a potential treatment for solid

tumors by Arrys/Kyn Therapeutics.[36] Bristol-Myers Squibb and Ono Pharmaceuticals are currently carrying out phase 2 clinical trials investigating the anti-tumor effects of the selective hEP4-R antagonist ONO-4578/BMS 986310 after having observed a compound-related improvement in the response of the immunosuppressive tumor microenvironment in preclinical studies.[37] The structure of this compound has not yet been disclosed. But the structure of ONO-AE3-208, an aryl butanoic acid based EP4-R antagonist has been disclosed[38] and more recently a paper describing the co-crystal complex of this compound with the human EP4 receptor has been published.[39] This EP4-R structure (5YWY.pdb) revealed a charge-charge interaction between the negatively charged carboxylate group of ONO-AE3-208 and the positively charged Arg^{316}, a residue that is conserved within the seventh transmembrane domain of all eight members of the prostanoid receptor family. The confirmation of this complementary charge-charge interaction may well explain the frequent occurrence of acidic centers in EP4-R antagonists. Eisai discovered the pyrazole derivative E7046 and investigated the role of this EP4 antagonist in cancer immunotherapy.[40] Adlai Nortye Pharmaceuticals and Merck are currently conducting a phase 1 clinical study with E7046/AN0025 in combination with Keytruda®.[41] Furthermore, Rottapharm is in phase 2 clinical trials for the treatment of rheumatoid arthritis with CR-6086[42] and Asterand/BTG/Ariel Pharmaceuticals reported on a phase 2 study in migraine patients using BGC-20-1531.[43] Merck also pursued an EP4-R antagonist program which resulted in the carboxylic acid containing compound MF-766.[44] However, no developmental activities relating to this compound appear to have been reported to date (see figure 1 for published hEP4-R antagonist structures).

In this paper, we demonstrate that benzimidazole carboxylic acid derivatives represent a new class of highly potent, specific and selective EP4-R antagonists. We discovered **BAY 1316957**, a

full antagonist of the human EP4 receptor with good oral bioavailability and excellent activity in an *in vivo* model of abdominal pain.[45] We report on structure-activity relationships (SAR) obtained while progressing from the initial screening hit through the lead-optimization process to the final candidate with a special focus on the strategies and considerations we applied in order to optimize the physicochemical and pharmacokinetic properties for its safe and efficacious use *in vivo*.

RESULTS AND DISCUSSION

High throughput screening (HTS) of the Bayer compound library (see supporting information for details) led to the identification of the initial hit compound **1** with an IC_{50} of 286 nM in a cell-based, functional assay measuring antagonism of the human EP4 receptor. Compound **1** was found to be highly lipophilic ($logD_{pH7.5}$ = 4.1) and had low solubility (S_w kinetic < 0.1 mg/L). Subsequent hit to lead activities resulted in identification of compound **2**, in which the *N*-methyl carboxamide of **1** was replaced by a carboxylic acid moiety. This compound was highly potent, with an IC_{50} of 13 nM at the hEP4-R, logD was reduced (3.0) and solubility was improved (S_w kinetic = 3.2 mg/L). However, due to the insufficient pharmacokinetic properties of the compound (data not shown), only low exposures were achieved in animals and no efficacy in an *in vivo* mouse model could be shown. Therefore, compounds with an improved pharmacokinetic profile were targeted.

Initial SAR evaluation was directed towards modifications of substituents on the carbazole and/or benzimidazole nitrogen atoms. Variations of the substituent on the carbazole nitrogen did not lead to any significant improvements in potency relative to compound **2** (table 1). In contrast, modifications to the substituent on the benzimidazole-nitrogen (table 2) ultimately led to the

identification of compound **13**, containing a methoxyethyl moiety. Compound **13** not only maintained single-digit nanomolar potency at the human EP4 receptor as well as a slightly improved potency at the corresponding mouse receptor (IC_{50} = 81 nM) but was also less lipophilic ($logD_{pH7.5}$ = 2.0) than compound **2** and showed increased solubility (S_w kinetic = 137 mg/L). Furthermore this compound had a significantly improved pharmacokinetic (PK) profile in rodents (low clearance, moderate volume of distribution, intermediate half-life, moderate oral bioavailability in rat, see table 6 for further details). In the mouse, free concentrations above the mouse IC_{50} could be maintained for approximately 6 hours after oral dosing thus making compound **13** a suitable *in vivo* pharmacodynamic tool compound for further evaluation and an excellent starting point for further optimization.

For the intended once daily application in humans, a further increase of bioavailability and thus unbound exposure (*i.e.* raising free drug concentrations) was considered to be necessary. Physicochemical parameters crucial to improving absorption (*i.e.* aqueous solubility, permeability) and/or increasing metabolic stability to decrease clearance of drug[46] were addressed to achieve this objective. As solubility and permeability (Caco-2 cellular assay) were already quite high (table 3), further improvement in metabolic stability (**13** shows moderate metabolic stability in both, human and rat hepatocytes, see table 3) was the primary focus of subsequent medicinal chemistry optimization efforts. Investigation of the biotransformation pathways of **13** showed that the compound was predominantly metabolized by formation of an acyl glucuronide of the carboxylic acid moiety in hepatocytes of all species studied (human, rat, monkey and dog). It should be noted that O- and N-dealkylation and oxidation were also observed to a minor extent.

Two main strategies to attenuate or circumvent the rapid glucuronidation of the carboxylic acid moiety were adopted, *i.e.* (1) the replacement of the carboxylic acid by bioisosteres and (2) structural modifications aiming to suppress the glucuronidation pathway by steric and/or electronic effects.

Several bioisosteric replacements of the carboxylic acid moiety in **13** were tolerated and resulted in compounds with similar potency. However, this approach did not result in a useful path forward to compounds with an improved overall profile. In general these changes resulted in insufficient levels of metabolic stability and/or permeability and/or solubility (table 3). For example, oxadiazolones **18** and **19** show very poor aqueous solubilities (< 1 mg/L) limiting their use for subsequent *in vivo* profiling. On the other hand, tetrazole **20** as well as acyl sulfonamides **22** and **25** all display low permeability and high efflux ratios and could not be considered for further progression as a result. Given the limited success obtained with bioisosteric replacements of the carboxylic acid moiety in **13,** the approach to overcome the low metabolic stability caused by intensive glucuronidation of compound **13** was investigated next.

Drugs containing carboxylic acids are highly susceptible to glucuronidation by uridine 5'-diphospho-glucuronosyltransferase (UDP-glucoronyltransferase, UGT) isoforms[47] (in-house studies demonstrated that, in this case, the main UGT involved in glucuronidation of compound **13** was UGT isoform 1A1 (UGT1A1; data not shown). By analyzing ~1800 nucleophiles Sorich *et al.* (2006)[47] showed that glucuronidation by human UGTs can be statistically predicted using just three variables (a Boolean variable indicating whether an aromatic ring was attached to the nucleophilic atom, the partial charge of the nucleophilic atom, and the atom-centered Fukui function), suggesting that the local structure of the nucleophilic atom is a key predictor for enzyme activity.

Having both, the catalytic mechanism for O-glucuronidation proposed by Dong *et al.* (2012)[48,49] as well as the influence of the local environment as described by Sorich et al. (2006)[47] in mind, we reasoned that we should be able to identify sites within the compound where appropriate modifications could likely impair or even prevent glucuronidation of the drug (figure 3). The so-called "serine hydrolase-like mechanism" involves a catalytic dyad of two key histidine and aspartic acid residues. The catalytic histidine abstracts a proton from the drug's carboxylic acid group to facilitate a nucleophilic attack at the C1 carbon of the glucuronic acid ring in uridine-5'-diphospho-α-D-glucuronic acid (UDPGA). Thus, introduction of small substituents in close proximity to the carboxylate moiety (red circle, as in compound **28**) was anticipated to prohibit glucuronidation either by clashing with the protein or the co-factor or by changing the orientation and accessibility of the nucleophilic oxygen atom. This hypothesis was tested by preparing EP4-antagonists containing small substituents in the vicinity of the carboxylate group – keeping in mind however, that although an X-ray structure of the C-terminal domain of the human UGT isoform 2B7 has been resolved,[50] detailed structural information about the complete substrate binding site (including the N-terminus) of other human UGTs is still missing. Clearly a number of additional factors may influence glucuronidation of **13** by human UGTs. In particular the actual UGT isoform involved determines the relevant protein-substrate interaction and final catalytic mechanism.[47,50]

In the first instance, methyl, chloro and fluoro were introduced as small substituents at both *ortho*- positions of the carboxylic acid moiety (table 4).

4-substituted benzimidazoles (R^4) displayed higher potency values when compared to their 6-substituted (R^6) analogs. Methyl-substituted derivative **28** combined low double-digit nanomolar potency with a higher metabolic stability in human and rat hepatocytes when compared to

compound **13**, thus supporting the hypothesis outlined above. Chloro-, and fluoro-substitutions in particular provided compounds with even better potency compared to methyl-substitution. However, Caco-2 permeability decreased and efflux ratios increased with halo-substituents. Compound **28** had the best overall balance of properties and was selected for further characterization. The *in vivo* rat PK profile nicely reflected the *in vitro* observations, showing low clearance, a long half-life, and a high oral bioavailability of 92% (table 6).

By comparing the metabolite profiles of the methylated compound **28** and the unsubstituted analog **13**, it was found that glucuronidation was still the major clearance pathway for **28** in human and rat hepatocytes. However, a significant attenuation of the glucuronide formation was observed for **28** on comparing the ratio parent drug / acyl glucuronide with those of the lead compound **13** (see figure 4).

Identification of BAY 1316957

With the favorable pharmacokinetic profile compound **28** in hand, a further SAR expansion around the carbazole part of the lead structure was undertaken (table 5), with the aim to improve the hEP4-R potency to compensate the 5-fold loss compared to **13**. We found that the introduction of small substituents at positions 5 - 8 of the carbazole moiety (R^{a2}, R^{a4}, R^{a5} in table 5) was generally well tolerated. Highly potent hEP4-R antagonists were obtained in most cases and the generally favorable pharmacokinetic and physicochemical profiles associated with parent compound **28** were maintained for the most of the compounds. Small lipophilic substituents as R^{a4} provided compounds displaying very favorable and balanced potency values across multiple species (human, rat, mouse, cynomolgus monkey, data not shown). On consideration of the overall preclinical profile (pharmacodynamics, pharmacokinetic and safety properties) as well as

synthetic accessibility and cost of goods, compound **32** (**BAY 1316957**) was selected for in-depth characterization as a potential drug candidate for oral administration.

Table 6 summarizes the progressive improvement of pharmacokinetic parameters during the optimization from lead compound **13** to the optimized lead **28** and the final candidate **32.** As expected, compound **32** showed a very similar PK profile in rats compared to compound **28** with a low clearance, long half-life and high bioavailability (see table 6). Furthermore, no alerts were raised with regard to clinically relevant potential for drug-drug interactions (data not shown). Investigation of the metabolic pathways of **32** in human, rat, mouse, dog and monkey hepatocytes revealed that the formation of the acyl glucuronide was also the common and predominant route of biotransformation, mainly catalyzed by UGT1A1 and to a lesser extent by UGT1A3. No metabolites, but only unchanged **32** was detected in rat plasma after oral and intravenous administration to rats. In rat excreta (bile), the expected acylglucuronide metabolite was observed as the main elimination product after intraveneous administration of **32** to bile-duct cannulated rats, which was found to be partly cleaved back to **32** in the faeces. This *in vivo* data confirmed the observations from the *in vitro* metabolism studies and indicated that the direct glucuronidation was the main elimination pathway of compound **32**.

Chemistry

The benzimidazoles **42** were synthesized as shown in Scheme 1 with the key step being the condensation of a phenylene diamine **40** with a carboxylic acid or a carbazole aldehyde (using sodium metabisulfite as oxidant) **41** to form benzimidazole **42.** Subsequently, Z could then be converted to the final benzimidazole carboxylic acid by saponification of the corresponding ester or via an alkoxy carbonylation / saponification sequence from the corresponding bromine derivative, respectively. Alternatively, Z could be developed to the bioisosteric replacements of the carboxylic acid using methods described in the literature (compounds **18 – 25**, syntheses described in the supporting information). Scheme 2 describes the general approach to accessing phenylene diamine and carbazole building blocks which are not commercially available. The phenylene diamines **40** were synthesized via reduction of the corresponding nitro anilines **45**. Two synthetic pathways for obtaining such nitro anilines are described. Substituent $\mathbf{R^1}$ was introduced either by aromatic nucleophilic substitution of the corresponding chloro precursor **43** with the respective amine or by reductive amination of a nitro aniline **44** with the appropriate aldehyde. N-alkylated carbazole carboxylic acids of type **41** were obtained using a modified strategy known in the literature.[51] Intermediates of types **46** and **47** were coupled by Suzuki reaction to afford intermediates **48** which were subsequently cyclized by intramolecular nucleophilic aromatic substitution of a fluorine atom[52] to afford carbazole based compounds **49**. Depending on reaction conditions the acid (**Y** = COOH) or the corresponding carboxylic acid ester (**Y** = C(O)OAlk) was formed in this step. Alkylation reactions at the NH and saponification, if necessary (when **Y** = C(O)OAlk), then afforded the desired acids of type **41**.

Compound **32** specifically was synthesized as shown in more detail in Scheme 3. Nitration of 4-chloro-2-methyl-benzoic acid **50** provided a 1:3 mixture of the 3-NO_2 and 5-NO_2 derivatives **51**

which were separated after step c. After formation of the methyl ester **52** methoxyethylamine was used to displace the chlorine and form intermediate **53**. Single regioisomer **53** was then reduced to yield phenylene diamine **54** as a key intermediate for the benzimidazole formation. Carbazole aldehyde **57** was obtained in a two-step procedure starting from commercially available carbazole **55** via bromination (with N-bromosuccinimide to afford **56**) and palladium (0)-catalyzed cross coupling reaction using methyl boronic acid. Cyclization of phenylene diamine **54** and carbazole aldehyde **57** with sodium metabisulfite gave benzimidazole methyl ester **58**, which was saponified to give the corresponding free carboxylic acid **32**.

Pharmacology

Activity in cellular assays. The identification and optimization of potent and selective hEP4-R subtype specific inhibitors was continuously supported with the help of two in-house prostanoid receptor panels which, in addition to determining the antagonistic activity and selectivity of the test compounds at the human EP4-, prostaglandin E2 subtype 2 (EP2)- and prostaglandin D2 subtype 1 (DP1)-R also included the compilation of species specificity data regarding the IC_{50} values obtained at the rat, mouse and cynomolgous monkey EP4 receptors (rEP4-, mEP4- and cEP4 receptor) - see experimental section for assay details and supporting information for detailed results regarding the key properties of lead compound **13** and **BAY 1316957** (**32**). In all cases cell-based, functional *in vitro* assays and agonist-induced generation of cyclic adenosine monophosphate (cAMP) were used to characterize the antagonistic activity of compounds with the help of a non-radioactive assay based on the competition between the endogenous second messenger and a fluorescently labeled cAMP-d2 donor for binding to an Europium cryptate labeled anti-cAMP antibody (cAMP-HTRF assay; CisBio International). Frozen cells[53] and

identical, mix and measure assay formats were used in both, the actual ultra-high-throughput screening assay (1536-well, generating > 100 000 data points / day) and the 384-well based assay, miniaturized dose response follow-up studies for the human receptors. Compounds antagonizing both hEP4- and hEP2-R signaling were found and excluded in early project phases only - even though inhibition of hEP2-R activity was hardly being detected. In marked contrast to a previous hEP2-R antagonist project[55] no interference with hDP-R activity was detected in the course of the continuous panel profiling activities at all. Cellular assays addressing the antagonization of agonist induced EP4-R activity in other species also used the 384-well format and the same detection technology.

Reduction of 16,16-dimethyl prostaglandin E2 ($dmPGE_2$) induced pain behavior in the rat. The employed dmPGE2 pain model involves a single injection of dmPGE2 into the hind paw of the animal.[53] The related pain associated behavior is measured as withdrawal reaction of the paw to increasing mechanical stimulation. Intraplantar administration of dmPGE2 (10 µg) produced significant mechanical allodynia approximately 30 minutes after injection in the vehicle treated animals in comparison to baseline measured on the day before. Oral administration of **BAY 1316957** (**32**) (5 mg/kg) 60 minutes prior to dmPGE2 injection significantly increased paw withdrawal thresholds assessed 30 minutes post- administration of dmPGE2 in comparison to the vehicle treated group (figure 5), suggesting a profound reduction of dmPGE2 induced pain. This effect stands in line with the expected profile of a potent and effective EP4 receptor antagonist.

Activity in off-target assays. In addition to inhouse panel profiling compounds selected for further development were subjected to comprehensive profiling studies including both, binding- and functional assays outsourced to and performed by contract research organizations in order to become aware of and to eliminate any potential and unanticipated off-target effects early on (see

supporting information for the results obtained regarding lead compound **13** and the final candidate **BAY 1316957 (32)).** Taken together, great care was taken to increase drug safety by proving both, hEP-4 R receptor selectivity and the absence of any cross reactivity with selected members of several therapeutic target classes.

Conclusion

We showed that benzimidazolyl carbazoles represent a new class of highly potent and selective hEP4-R receptor antagonists. Optimization of the HTS hit **1** resulted in the more potent lead compound **13**, displaying a carboxylic acid moiety. However, direct glucuronidation of this carboxylic acid moiety was identified as the critical metabolic pathway which needed to be attenuated to achieve a favorable pharmacokinetic profile. Careful SAR investigation guided by rational design identified substitution adjacent to the carboxylic acid as an appropriate way to accomplish reduction of glucuronidation. Further potency optimization yielded **BAY 1316957 (32)** as drug candidate. With its excellent *in vitro* and *in vivo* efficacy and its superior pharmacokinetic profile, **BAY 1316957** is suitable for further development for the treatment of endometriosis in women.

EXPERIMENTAL SECTION

Chemistry. General. Commercially available reagents and anhydrous solvents were used without further purification. All air- and moisture-sensitive reactions were carried out in oven-dried (at 120 °C) glassware under an inert atmosphere of argon. The purity of all final compounds was ≥95%, determined by LCMS and ^{1}H-NMR. Reactions were monitored by TLC and ultra performance liquid chromatography (UPLC) analysis with a Waters Acquity UPLC MS

Single Quad system; column: Acquity UPLC BEH C18 1.7 µm, 50 × 2.1 mm; eluent A: Water (H_2O) + 0.2 vol% aqueous (aq.) ammonia (NH_3, 32%), eluent B: acetonitrile (MeCN); gradient: 0–1.6 min 1–99% B, 1.6–2.0 min 99% B; flow: 0.8 mL/min; temperature: 60 °C; diode array detector (DAD) scan: 210–400 nm. Flash chromatography was carried out using a Biotage® Isolera™ One system with 200–400 nm variable detector, using Biotage® SNAP KP-Sil or KP-NH cartridges. Preparative HPLC was carried out with a Waters AutoPurification MS Single Quad system; column: Waters XBridge C18 5 µm, 100 × 30 mm; eluent A: H_2O + 0.2 vol% aq NH_3 (32%), eluent B: MeCN; gradient: 0–5.5 min 5–100% B; flow: 70 mL/min; temperature: 25 °C; DAD scan: 210–400 nm. Analytical TLC was carried out on aluminum-backed plates coated with Merck Kieselgel 60 F254, with visualization under UV light at 254 nm. All NMR spectra were recorded on Bruker Avance III HD spectrometers. ^{1}H NMR spectra were obtained at 300, 400, 500, or 600 MHz and referenced to the residual solvent signal (2.49 ppm for DMSO-d_6). ^{13}C NMR spectra were obtained at 101 or 151 MHz and also referenced to the residual solvent signal (39.7 ppm for DMSO-d_6). All spectra were obtained at ambient temperature (22 ± 1 °C). ^{1}H NMR data are reported as follows: chemical shift (δ) in ppm, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constant(s) (Hz), and integration. Mass spectra were recorded on LC-MS instruments: (i) Waters Acquity UPLC MS Single Quad system; column: Kinetex 2.6 µm, 50 × 2.1 mm, or (ii) Agilent 1290 UPLC MS 6230 TOF system; column: BEH C18 1.7 µm, 50 × 2.1 mm; eluent A: H_2O + 0.05% formic acid (99%), eluent B: MeCN + 0.05% formic acid (99%). Fragment ions are reported as m/z values with relative intensities (%) in parentheses. High-resolution mass spectra were recorded on a Xevo® G2-XS QTof (Waters) instrument.

1-(Cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-*N*-methyl-1*H*-benzimidazole-5-carboxamide (1). 100 mg (0.24 mmol) of 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**2**), 38 mg (0.37 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 56 mg (0.37 mmol) of 1-hydroxyl-1*H*-benzotriazole (HOBT hydrate), 221 mg (1.71 mmol) of *N*, *N*-diisopropylethylamine (DIPEA) and 0.61 mL 2 M methylamine in 4.6 mL *N,N*-dimethylformamide (DMF) were stirred at rt for 3 days. The reaction mixture was then concentrated, DMSO added, the mixture filtered and the filtrate separated by preparative HPLC. In this way, 60 mg (58%) of the title compound **1** were obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, *J*=1.26 Hz, 1H), 8.43 (q, *J*=4.04 Hz, 1H), 8.30 (d, *J*=7.58 Hz, 1H), 8.21 (d, *J*=1.01 Hz, 1H), 7.67-7.89 (m, 5H), 7.53 (dt, *J*=1.14, 7.64 Hz, 1H), 7.24-7.29 (m, 1H), 4.53 (q, *J*=7.07 Hz, 2H), 4.35 (d, *J*=6.82 Hz, 2H), 2.84 (d, *J*=4.29 Hz, 3H), 1.38 (t, *J*=7.07 Hz, 3H), 1.01-1.12 (m, 1H), 0.32-0.40 (m, 2H), 0.13-0.19 (m, 2H); HRMS (ESI, $[M+H]^+$): calc.: 423.2185, found: 423.2182.

1-(Cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (2). (a) Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**59**). 25.7 g (135 mmol) of sodium disulfite were dissolved in 60 mL of water and treated with a solution of 14.8 g (66 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde in 135 mL of tetrahydrofuran (THF). 20 g (120 mmol) of methyl 3, 4-diaminobenzoate in 90 mL of THF were then added and the mixture was heated to reflux for 3 h and stirred for 15 h with cooling to room temperature (RT). 150 mL of saturated sodium hydrogencarbonate solution were added to the reaction mixture, it was extracted several times with dichloromethane, and the collected organic phases were dried using sodium sulfate and evaporated to dryness. The residue was taken up in dichloromethane and brought to crystallization. 20.56 g (84%) of the title compound **59** were thus obtained. ^{1}H NMR

(300 MHz, DMSO-d_6) δ 13.20 (br d, *J*=7.72 Hz, 1H), 9.01 (s, 1H), 8.00-8.46 (m, 3H), 7.61-7.91 (m, 4H), 7.53 (t, *J*=7.63 Hz, 1H), 7.29 (t, *J*=7.44 Hz, 1H), 4.52 (q, *J*=7.03 Hz, 2H), 3.34 (s, 3H), 1.36 (t, *J*=7.06 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 410.1869, found: 410.1865.

(b) Methyl 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**60**). 2.429 g (60.74 mmol) of sodium hydride (NaH, 60%) were first added to a solution of 20.4 g (4.1 mmol) of methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**59**) in 510 mL of DMF under argon, subsequently stirred at RT for 30 min and then treated with 6.43 mL (66.26 mmol) of (bromomethyl)-cyclopropane. After 30 h, saturated sodium hydrogencarbonate solution was added and the mixture was extracted several times with dichloromethane. The collected organic phases were washed with water, dried using sodium sulfate and concentrated. The residue was first purified by chromatography on silica gel (hexane/ethyl acetate 1:1) and the crude product (16.79 g) thus obtained was separated by preparative HPLC. In this way, 7.04 g (30%) of the title compound **60** and 5.96 g (25%) of the isomeric methyl 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-6-carboxylate were obtained. Methyl 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**60**) ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (d, *J*=1.32 Hz, 1H), 8.27-8.35 (m, 2H), 7.78-7.96 (m, 4H), 7.69 (d, *J*=8.29 Hz, 1H), 7.49-7.57 (m, 1H), 7.27 (t, *J*=7.25 Hz, 1H), 4.53 (q, *J*=6.97 Hz, 2H), 4.38 (d, *J*=6.59 Hz, 2H), 3.90 (s, 3H), 1.38 (t, *J*=7.06 Hz, 3H), 0.97-1.12 (m, 1H), 0.31-0.42 (m, 2H), 0.11-0.22 (m, 2H). Methyl 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-6-carboxylate ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (d, *J*=1.13 Hz, 1H), 8.35 (s, 1H), 8.30 (d, *J*=7.80 Hz, 1H), 7.66-7.93 (m, 5H), 7.52 (t, *J*=7.16 Hz, 1H), 7.26 (t, *J*=7.54 Hz, 1H), 4.53 (q, *J*=6.91 Hz, 2H), 4.44 (d,

J=6.78 Hz, 2H), 3.91 (s, 3H), 1.38 (t, *J*=7.06 Hz, 3H), 1.00-1.14 (m, 1H), 0.32-0.43 (m, 2H), 0.16 (q, *J*=4.71 Hz, 2H).

(c) 1-(Cyclopropylmethyl)-2-(9-ethyl-9H-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**2**). 7.04 g (16.62 mmol) of methyl 1-(cyclopropylmethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**60**) were dissolved in a mixture of 250 mL of ethanol, treated with 133 mL of 1.0 M sodium hydroxide (NaOH) solution and heated to 80 °C for 20 h. After cooling to RT, the mixture was acidified to pH 2 with 1 M hydrochloric acid (HCl), extracted several times with ethyl acetate and the combined organic phases were concentrated to dryness. 4.99 g (73%) of the title compound **2** were thus obtained. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.78 (br s, 1H), 8.66 (d, *J*=1.13 Hz, 1H), 8.27-8.33 (m, 2H), 7.80-7.98 (m, 4H), 7.70 (d, *J*=8.29 Hz, 1H), 7.54 (t, *J*=7.62 Hz, 1H), 7.28 (t, *J*=7.16 Hz, 1H), 4.54 (q, *J*=6.91 Hz, 2H), 4.40 (d, *J*=6.78 Hz, 2H), 1.38 (t, *J*=7.06 Hz, 3H), 1.01-1.15 (m, 1H), 0.33-0.43 (m, 2H), 0.13-0.23 (m, 2H).

1-(Cyclopropylmethyl)-2-(9-methyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (3). (a) 3-Amino-4-[(cyclopropylmethyl)amino]benzoic acid (**61**). In analogy to 3-amino-4-[(2-methoxyethyl)amino]benzoic acid **(76)**, 3-amino-4-[(cyclopropylmethyl)amino]benzoic acid **61** was obtained from ethyl 4-chloro-3-nitrobenzoate and 1-cyclopropylmethanamine in three steps. 1H NMR (300 MHz, DMSO-d_6) δ 9.92-12.86 (m, 1H), 7.18-7.29 (m, 2H), 6.46 (d, *J*=8.29 Hz, 1H), 5.00-5.80 (m, 3H), 2.98 (d, *J*=6.59 Hz, 2H), 1.02-1.15 (m, 1H), 0.41-0.57 (m, 2H), 0.18-0.31 (m, 2H); HRMS (ESI, $[M+H]^+$): calc.: 396.1712, found: 396.1707.

(b) 1-(Cyclopropylmethyl)-2-(9-methyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**3**). In analogy to example **13** (route A), 55 mg (28%) of the title compound **3** were obtained from 101 mg (0.49 mmol) of 9-methyl-9*H*-carbazole-3-carbaldehyde and 150 mg (0.73 mmol) of

3-amino-4-[(cyclopropylmethyl)amino]benzoic acid **(61)**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.27-13.28 (m, 1H), 8.64 (d, *J*=1.13 Hz, 1H), 8.27 (d, *J*=1.13 Hz, 2H), 7.75-7.96 (m, 4H), 7.68 (d, *J*=8.29 Hz, 1H), 7.54 (t, *J*=7.63 Hz, 1H), 7.27 (t, *J*=7.54 Hz, 1H), 4.37 (d, *J*=6.78 Hz, 2H), 3.97 (s, 3H), 0.98-1.12 (m, 1H), 0.27-0.44 (m, 2H), 0.13 (q, *J*=4.90 Hz, 2H).

1-(Cyclopropylmethyl)-2-(9-propyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (4). (a) 9-Propyl-9*H*-carbazole-3-carbaldehyde (**62**). 200 mg (1.02 mmol) of 9*H*-carbazole-3-carbaldehyde in DMF were added to 45 mg of NaH (60%) in 1 mL of toluene at 0°C. After 30 minutes 0.104 mL of 1-bromopropane (1.23 mmol) were added and the mixture was stirred at RT overnight. Water was added to the reaction mixture, the mixture was extracted several times with ethyl acetate and the combined organic phases were concentrated to dryness. In this way, 225 mg (92%) of crude material of the title compound **62** were obtained which was used immediately in the next step.

(b) 1-(Cyclopropylmethyl)-2-(9-propyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**4**). In analogy to example **13** (route A), 7.5 mg (6.9%) of the title compound **4** were obtained from 57 mg (0.24 mmol) of aldehyde **62** and 100 mg (0.48 mmol) of benzoic acid **61**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.67 (s, 1H), 8.52 (s, 1H), 8.14 (t, *J*=8.38 Hz, 2H), 7.85 (dd, *J*=1.41, 8.57 Hz, 1H), 7.49-7.62 (m, 4H), 7.29-7.34 (m, 1H), 4.36 (t, *J*=7.06 Hz, 2H), 4.29 (d, *J*=6.59 Hz, 2H), 1.93-2.06 (m, 2H), 1.23 (br s, 1H), 1.03 (t, *J*=7.44 Hz, 3H), 0.44-0.56 (m, 2H), 0.21 (q, *J*=4.96 Hz, 2H), COOH not stated; HRMS (ESI, $[M+H]^+$): calc.: 424.2025, found: 424.2022.

1-(Cyclopropylmethyl)-2-[9-(cyclopropylmethyl)-9*H*-carbazol-3-yl]-1*H*-benzimidazole-5-carboxylic acid (5). (a) 9-(Cyclopropylmethyl)-9*H*-carbazole-3-carbaldehyde (**63**). In analogy to

aldehyde **62**, 233 mg (91%) of crude material of the title compound **63** were obtained from 200 mg (1.02 mmol) of 9*H*-carbazole-3-carbaldehyde and 0.119 mL of (bromomethyl)cyclopropane (1.23 mmol) and was used immediately in the next step.

(b) 1-(Cyclopropylmethyl)-2-[9-(cyclopropylmethyl)-9*H*-carbazol-3-yl]-1*H*-benzimidazole-5-carboxylic acid (**5**). In analogy to example **13** (route A), 13.9 mg (13%) of the title compound **5** were obtained from 60 mg (0.24 mmol) of aldehyde **63** and 100 mg (0.48 mmol) of benzoic acid **61**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.67 (d, *J*=1.13 Hz, 1H), 8.52 (d, *J*=1.32 Hz, 1H), 8.09-8.19 (m, 2H), 7.85 (dd, *J*=1.60, 8.38 Hz, 1H), 7.47-7.62 (m, 4H), 7.29-7.34 (m, 1H), 4.31 (dd, *J*=6.59, 8.85 Hz, 4H), 1.32-1.46 (m, 1H), 1.17-1.31 (m, 1H), 0.50-0.65 (m, 4H), 0.47 (q, *J*=5.02 Hz, 2H), 0.22 (q, *J*=5.09 Hz, 2H), COOH not stated; HRMS (ESI, $[M+H]^+$): calc.: 436.2025, found: 436.2025.

2-[9-(Cyclobutylmethyl)-9*H*-carbazol-3-yl]-1-(cyclopropylmethyl)-1*H*-benzimidazole-5-carboxylic acid (6). (a) 9-(Cyclobutylmethyl)-9*H*-carbazole-3-carbaldehyde (**64**). In analogy to aldehyde **62**, 299 mg (110%) of crude material of the title compound **64** were obtained from 200 mg (1.02 mmol) of 9*H*-carbazole-3-carbaldehyde and 0.104 mL of (bromomethyl)cyclobutane (1.23 mmol) and used immediately in the next step.

(b) 2-[9-(Cyclobutylmethyl)-9*H*-carbazol-3-yl]-1-(cyclopropylmethyl)-1*H*-benzimidazole-5-carboxylic acid (**6**). In analogy to example **13** (route A), 22 mg (19%) of the title compound **6** were obtained from 64 mg (0.24 mmol) of aldehyde **64** and 100 mg (0.48 mmol) of benzoic acid **61**. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.68 (s, 1H), 8.51 (d, *J*=1.25 Hz, 1H), 8.11-8.17 (m, 2H), 7.85 (dd, *J*=1.51, 8.53 Hz, 1H), 7.47-7.62 (m, 4H), 7.28-7.34 (m, 1H), 4.40 (d, *J*=7.03 Hz, 2H), 4.29 (d, *J*=6.53 Hz, 2H), 2.95-3.05 (m, 1H), 2.02-2.13 (m, 2H), 1.86-1.98 (m, 4H),

1.19-1.28 (m, 1H), 0.48-0.59 (m, 2H), 0.22 (q, *J*=5.02 Hz, 2H), COOH not stated; HRMS (ESI, $[M+H]^+$): calc.: 450.2182, found: 450.2176.

1-(Cyclopropylmethyl)-2-[9-(2-methoxyethyl)-9*H*-carbazol-3-yl]-1*H*-benzimidazole-5-carboxylic acid (7). (a) 9-(2-Methoxyethyl)-9*H*-carbazole-3-carbaldehyde (**65**). In analogy to aldehyde **62**, 770 mg (119%) of crude material of the title compound **65** were obtained from 500 mg (2.56 mmol) of 9*H*-carbazole-3-carbaldehyde and 1.479 mL of 1-bromo-2-methoxyethane (3.07 mmol). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.74-8.77 (m, 1H), 8.29 (d, *J*=7.54 Hz, 1H), 7.92-7.97 (m, 1H), 7.79 (d, *J*=8.48 Hz, 1H), 7.71 (d, *J*=8.29 Hz, 1H), 7.53 (dt, *J*=1.13, 7.72 Hz, 1H), 7.27-7.37 (m, 1H), 4.64 (t, *J*=5.18 Hz, 2H), 3.74 (t, *J*=5.18 Hz, 2H), 3.17 (s, 3H).

(b) 1-(Cyclopropylmethyl)-2-[9-(2-methoxyethyl)-9*H*-carbazol-3-yl]-1*H*-benzimidazole-5-carboxylic acid (**7**). In analogy to example **13** (route A), 18 mg (15%) of the title compound **7** were obtained from 67 mg (0.26 mmol) of aldehyde **65** and 109 mg (0.53 mmol) of benzoic acid **51**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 12.33-12.91 (m, 1H), 8.67 (s, 1H), 8.51 (d, *J*=1.32 Hz, 1H), 8.10-8.19 (m, 2H), 7.85 (dd, *J*=1.60, 8.57 Hz, 1H), 7.46-7.66 (m, 4H), 7.28-7.37 (m, 1H), 4.57 (t, *J*=5.84 Hz, 2H), 4.29 (d, *J*=6.59 Hz, 2H), 3.85 (t, *J*=5.84 Hz, 2H), 3.34 (s, 3H), 1.16-1.35 (m, 1H), 0.48-0.61 (m, 2H), 0.22 (q, *J*=5.09 Hz, 2H); HRMS (ESI, $[M+H]^+$): calc.: 440.1974, found: 440.1972.

1-(2-Cyclopropylethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (8). (a) Ethyl 3-amino-4-[(2-cyclopropylethyl)amino]benzoate (**66**). In analogy to ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)**, ethyl 3-amino-4-[(2-cyclopropylethyl)amino]benzoate **(66)** was obtained from ethyl 4-chloro-3-nitrobenzoate and 2-cyclopropylethanamine in two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.07-7.15 (m, 2H), 6.36 (d, *J*=8.34 Hz, 1H), 5.02-5.17

(m, 1H), 4.64-4.68 (m, 1H), 4.11 (q, *J*=7.16 Hz, 2H), 3.05-3.14 (m, 2H), 1.43 (q, *J*=7.07 Hz, 2H), 1.18 (t, *J*=7.07 Hz, 3H), 0.66-0.84 (m, 1H), 0.31-0.39 (m, 2H), -0.01-0.05 (m, 2H).

(b) Ethyl 1-(2-cyclopropylethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**67**). In analogy to example **13** (route B), 37 mg (22%) of the title compound **67** were obtained from 54 mg (0.24 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 90 mg (0.36 mmol) of benzoate **66**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (d, *J*=1.32 Hz, 1H), 8.28-8.32 (m, 2H), 7.87-7.94 (m, 2H), 7.79-7.84 (m, 2H), 7.70 (d, *J*=8.29 Hz, 1H), 7.50-7.56 (m, 1H), 7.27 (t, *J*=7.54 Hz, 1H), 4.49-4.58 (m, 4H), 4.36 (q, *J*=7.16 Hz, 2H), 1.61 (q, *J*=6.97 Hz, 2H), 1.34-1.41 (m, 6H), 0.40-0.48 (m, 1H), 0.17-0.23 (m, 2H), -0.17 (q, *J*=4.77 Hz, 2H).

(c) 1-(2-Cyclopropylethyl)-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**8**). In analogy to example **13** (route B), 2.1 mg (5.5%) of the title compound **8** were obtained from 37 mg (0.08 mmol) of ethyl ester **57**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27-12.85 (m, 1H), 8.54 (s, 1H), 8.21 (d, *J*=7.45 Hz, 1H), 8.18 (s, 1H), 7.79-7.87 (m, 2H), 7.69-7.76 (m, 2H), 7.61 (d, *J*=8.08 Hz, 1H), 7.44 (t, *J*=7.33 Hz, 1H), 7.19 (t, *J*=7.20 Hz, 1H), 4.40-4.50 (m, 4H), 1.54 (q, *J*=6.82 Hz, 2H), 1.29 (br t, *J*=7.07 Hz, 3H), 0.75-0.79 (m, 1H), 0.30-0.41 (m, 2H), 0.09-0.15 (m, 2H).

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-isopropyl-1*H*-benzimidazole-5-carboxylic acid (9). (a) Ethyl 3-amino-4-(isopropylamino)benzoate (**68**). In analogy to ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)**, ethyl 3-amino-4-(isopropylamino)benzoate **(68)** was obtained from ethyl 4-chloro-3-nitrobenzoate and isopropylamine in two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.17-7.26 (m, 2H), 6.46 (d, *J*=8.59 Hz, 1H), 5.01 (br s, 2H), 4.15-4.23 (m,

2H), 3.65 (septet, *J*=6.32 Hz, 1H), 1.26 (t, *J*=7.07 Hz, 3H), 1.18 (d, *J*=6.32 Hz, 6H), NH not stated.

(b) Ethyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-isopropyl-1*H*-benzimidazole-5-carboxylate (**69**). In analogy to example **13** (route B), 127 mg (68%) of the title compound **69** were obtained from 60 mg (0.27 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 90 mg (0.40 mmol) of benzoate **58**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, *J*=1.32 Hz, 1H), 8.26-8.33 (m, 2H), 7.80-8.00 (m, 3H), 7.68-7.76 (m, 2H), 7.49-7.58 (m, 1H), 7.22-7.30 (m, 1H), 4.88 (quintet, *J*=6.92 Hz, 1H), 4.48-4.64 (m, 2H), 4.29-4.42 (m, 2H), 1.64 (d, *J*=6.97 Hz, 6H), 1.33-1.41 (m, 6H).

(c) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-isopropyl-1*H*-benzimidazole-5-carboxylic acid (**9**). In analogy to example **13** (route B), 34 mg (33%) of the title compound **9** were obtained from 107 mg (0.25 mmol) of ethyl ester **69**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.61-13.85 (br s, 1H), 8.67 (s, 1H), 8.26-8.38 (m, 3H), 8.10 (dd, *J*=1.32, 8.85 Hz, 1H), 7.85-8.01 (m, 2H), 7.76 (d, *J*=8.29 Hz, 1H), 7.59 (t, *J*=7.44 Hz, 1H), 7.33 (t, *J*=7.25 Hz, 1H), 5.01 (td, *J*=6.76, 13.80 Hz, 1H), 4.58 (q, *J*=6.78 Hz, 2H), 1.74 (d, *J*=6.78 Hz, 6H), 1.39 (t, *J*=7.06 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 398.1869, found: 398.1866.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-5-carboxylic acid (10). (a) Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**70**). In analogy to the synthesis of intermediate **60**, 2.9 g (18%) of benzimidazole **70** were obtained from 15 g (40.6 mmol) of intermediate **59** and 7.58 mL (121.8 mmol) of methyl iodide in 18% yield together with its regioisomer methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-6-carboxylate (2.54 g, 16%). Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-5-carboxylate (**70**) ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.57 (d, *J*=1.32 Hz, 1H), 8.14-8.24

(m, 2H), 8.05 (dd, *J*=1.51, 8.48 Hz, 1H), 7.90 (d, *J*=8.20 Hz, 1H), 7.85 (d, *J*=8.85 Hz, 1H), 7.42-7.65 (m, 3H), 7.32 (t, *J*=6.99 Hz, 1H), 4.45 (q, *J*=7.28 Hz, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 1.51 (t, *J*=7.16 Hz, 3H). Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-6-carboxylate ^{1}H NMR (600 MHz, CHLOROFORM-d) δ 8.56 (d, *J*=1.51 Hz, 1H), 8.18 (s, 1H), 8.16 (d, *J*=7.68 Hz, 1H), 8.04 (dd, *J*=1.51, 8.28 Hz, 1H), 7.89 (d, *J*=8.44 Hz, 1H), 7.84 (d, *J*=8.35 Hz, 1H), 7.51-7.56 (m, 2H), 7.45-7.49 (m, 1H), 7.29 (t, *J*=7.41 Hz, 1H), 4.44 (q, *J*=7.40 Hz, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 1.49 (t, *J*=7.15 Hz, 3H).

(b) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-methyl-1*H*-benzimidazole-5-carboxylic acid (**10**). In analogy to example **2**, 2.3 g (91%) of the title compound **10** were obtained from 2.5 g (6.52 mmol) of methyl ester **70** and 52 mL of 1N NaOH in 60 mL of ethanol/1,2-dichloroethane (1:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.88 (d, *J*=1.51 Hz, 1H), 8.31-8.39 (m, 2H), 8.05-8.19 (m, 3H), 7.95-8.03 (m, 1H), 7.77 (d, *J*=8.10 Hz, 1H), 7.59 (t, *J*=7.35 Hz, 1H), 7.35 (t, *J*=7.35 Hz, 1H), 4.58 (q, *J*=7.10 Hz, 2H), 4.18 (s, 3H), 1.38 (t, *J*=7.06 Hz, 3H), COOH not stated; HRMS (ESI, $[M+H]^+$): calc.: 370.1556, found: 370.1557.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(oxetan-3-ylmethyl)-1*H*-benzimidazole-5-carboxylic acid (11). (a) Ethyl 3-amino-4-[(oxetan-3-ylmethyl)amino]benzoate (**71**). In analogy to ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)**, intermediate **71** was obtained from ethyl 4-chloro-3-nitrobenzoate and 1-(oxetan-3-yl)methanamine in two steps. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35-7.42 (m, 1H), 7.26-7.35 (m, 2H), 6.49-6.60 (m, 1H), 5.38-5.66 (m, 1H), 4.69 (dd, *J*=6.03, 7.54 Hz, 2H), 4.24-4.39 (m, 4H), 3.40-3.44 (m, 2H), 3.17-3.29 (m, 2H), 1.28 (d, *J*=6.97 Hz, 3H).

(b) Ethyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(oxetan-3-ylmethyl)-1*H*-benzimidazole-5-carboxylate (**72**). In analogy to example **13** (route B), 45 mg (22%) of the title compound **72** were obtained from 59 mg (0.27 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 100 mg (0.40 mmol) of benzoate **71**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (d, *J*=0.94 Hz, 1H), 8.27-8.35 (m, 2H), 7.76-7.98 (m, 4H), 7.70 (d, *J*=8.29 Hz, 1H), 7.54 (t, *J*=7.86 Hz, 1H), 7.28 (t, *J*=7.44 Hz, 1H), 4.86 (d, *J*=7.35 Hz, 2H), 4.49-4.60 (m, 2H), 4.29-4.47 (m, 4H), 4.01-4.10 (m, 2H), 3.19-3.29 (m, 1H), 1.38 (q, *J*=6.78 Hz, 6H).

(c) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(oxetan-3-ylmethyl)-1*H*-benzimidazole-5-carboxylic acid (**11**). In analogy to example **13** (route B), 16 mg (39%) of the title compound **11** were obtained from 40 mg (0.09 mmol) of ethyl ester **72**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.26-8.35 (m, 2H), 8.04 (s, 2H), 7.88-8.01 (m, 2H), 7.74 (d, *J*=8.10 Hz, 1H), 7.56 (t, *J*=7.44 Hz, 1H), 7.31 (t, *J*=7.44 Hz, 1H), 4.95 (br d, *J*=7.35 Hz, 2H), 4.57 (q, *J*=6.84 Hz, 2H), 4.42 (dd, *J*=6.40, 7.54 Hz, 2H), 4.08 (t, *J*=6.12 Hz, 2H), 3.27-3.47 (m, 2H), 1.39 (t, *J*=7.06 Hz, 3H).

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(tetrahydrofuran-2-ylmethyl)-1*H*-benzimidazole-5-carboxylic acid (12). In analogy to the synthesis of example **2**, 17 mg (4%) of benzimidazole **12** were obtained from 200 mg (0.541 mmol) of intermediate **59** in 2 steps together with its regioisomer 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(tetrahydrofuran-2-ylmethyl)-1*H*-benzimidazole-6-carboxylic acid (17 mg, 4%). 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(tetrahydrofuran-2-ylmethyl)-1*H*-benzimidazole-5-carboxylic acid (**12**) ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.22-13.20 (m, 1H), 8.67 (d, *J*=1.25 Hz, 1H), 8.24-8.31 (m, 2H), 7.88-7.96 (m, 2H), 7.79 (dd, *J*=3.26, 8.53 Hz, 2H), 7.68 (d, *J*=8.28 Hz, 1H), 7.52 (t, *J*=7.28 Hz, 1H), 7.27 (t, *J*=7.53 Hz, 1H), 4.40-4.58 (m, 4H), 4.18-4.30 (m, 1H), 3.53-3.69 (m, 2H), 1.88-1.99 (m, 1H), 1.75 (quintet, *J*=7.09 Hz, 2H), 1.43-1.58 (m, 1H), 1.38 (t, *J*=7.15 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 440.1974, found: 440.1966.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (13). Route A: 4.78 g (25.19 mmol) of sodium disulfite were added to 11 mL of water and then treated with a solution of 2.5 g (11.20 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde in 25 mL of THF. 3.53 g (16.80 mmol) of 3-amino-4-[(2-methoxyethyl)amino]benzoic acid **(76)** in 10 mL of THF were then added, and the mixture was stirred at RT for 10 min. and subsequently heated to reflux for 2.5 h. After cooling, the reaction mixture was treated with 7.5 mL of water, acidified to pH 2 with 1 M HCl and extracted three times with dichloromethane. The collected organic phases were washed with water, dried with sodium sulfate and concentrated to dryness. The residue was suspended in ethyl acetate, 3.99 g (86%) of the title compound **13** were obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 8.68 (d, *J*=1.52 Hz, 1H), 8.25-8.29 (m, 2H), 7.88-7.98 (m, 2H), 7.79 (t, *J*=8.84 Hz, 2H), 7.69 (d, *J*=8.34 Hz, 1H), 7.53 (t, *J*=7.38 Hz, 1H), 7.27 (t, *J*=7.16 Hz, 1H), 4.48-4.64 (m, 4H), 3.73 (t, *J*=5.31 Hz, 2H), 3.11-3.19 (m, 3H), 1.38 (t, *J*=7.20 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 414.1818, found: 414.1808.

Route B: (a) Ethyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate (**77**). 1.2 g (6.30 mmol) of sodium disulfite, 0.625 g (2.80 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)** in 20 mL of THF/water (1:1) were heated to reflux for 2.5 h. After cooling, the reaction mixture was concentrated to dryness and purified by column chromatography (silica gel, hexane/ethyl acetate) to give 1.18 g (57%) of the title compound **77**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (d, *J*=1.32 Hz, 1H), 8.29 (s, 1H), 8.27 (d, *J*=8.81 Hz, 1H), 7.94 (dt, *J*=1.70, 8.19 Hz, 2H), 7.81 (dd, *J*=1.70, 8.48 Hz, 2H), 7.69 (d, *J*=8.29 Hz, 1H), 7.53 (t, *J*=7.58 Hz, 1H), 7.21-7.32 (m, 1H), 4.48-4.65 (m, 4H), 4.36 (q, *J*=7.16 Hz, 2H), 3.72 (t, *J*=5.18 Hz, 2H), 3.13 (s, 3H), 1.37 (t, *J*=7.06 Hz, 6H).

(b) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (**13**). 1.9 g (4.30 mmol) of ethyl ester **77** were dissolved in 20 mL of ethanol, treated with 3.0 mL of 2.0 M NaOH solution and heated to reflux for 1 h. After cooling to RT, the mixture was acidified to pH 2 with 2 M HCl, extracted several times with dichloromethane and the combined organic phases were concentrated to dryness, to give 1.70 g (86%) of the title compound **13**.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(3-methoxypropyl)-1*H*-benzimidazole-5-carboxylic acid (14). (a) Ethyl 3-amino-4-[(3-methoxypropyl)amino]benzoate (**78**). In analogy to ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)**, intermediate **78** was obtained from ethyl 4-chloro-3-nitrobenzoate and 3-methoxypropan-1-amine in two steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24 (dd, *J*=2.02, 8.34 Hz, 1H), 7.16-7.22 (m, 1H), 6.45 (d, *J*=8.59 Hz, 1H), 5.27 (br s, 2H), 4.19 (q, *J*=7.07 Hz, 2H), 3.41-3.46 (m, 2H), 3.25 (s, 3H), 3.12-3.18 (m, 2H), 1.75-1.88 (m, 2H), 1.23-1.29 (m, 3H), NH not specified.

(b) Ethyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(3-methoxypropyl)-1*H*-benzimidazole-5-carboxylate (**79**). In analogy to example **13** (route B), 100 mg (51%) of the title compound **79** were obtained from 59 mg (0.26 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 100 mg (0.40 mmol) of benzoate **78**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (d, *J*=1.32 Hz, 1H), 8.25-8.33 (m, 2H), 7.68-7.98 (m, 5H), 7.54 (t, *J*=7.62 Hz, 1H), 7.28 (t, *J*=7.44 Hz, 1H), 4.45-4.62 (m, 4H), 4.36 (q, *J*=7.03 Hz, 2H), 3.22 (t, *J*=5.75 Hz, 2H), 3.05 (s, 3H), 1.89-2.09 (m, 2H), 1.37 (t, *J*=7.06 Hz, 6H).

(c) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(3-methoxypropyl)-1*H*-benzimidazole-5-carboxylic acid (**14**). In analogy to example **13** (route B), 60 mg (64%) of the title compound **14** were obtained from 100 mg (0.22 mmol) of ethyl ester **79**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.60-12.93 (m,

1H), 8.63 (d, *J*=1.13 Hz, 1H), 8.29 (d, *J*=7.75 Hz, 1H), 8.27 (s, 1H), 7.88-7.96 (m, 2H), 7.79-7.84 (m, 1H), 7.67-7.76 (m, 2H), 7.53 (t, *J*=7.65 Hz, 1H), 7.27 (t, *J*=7.44 Hz, 1H), 4.47-4.58 (m, 4H), 3.20-3.26 (m, 2H), 3.06 (s, 3H), 1.99 (br d, *J*=7.16 Hz, 2H), 1.38 (t, *J*=7.06 Hz, 3H).

1-[2-(Dimethylamino)ethyl]-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (15). (a) Ethyl 3-amino-4-{[2-(dimethylamino)ethyl]amino}benzoate (**80**). In analogy to ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate **(75)**, intermediate **80** was obtained from ethyl 4-chloro-3-nitrobenzoate and N,N-dimethylethane-1,2-diamine in two steps.

(b) Ethyl 1-[2-(dimethylamino)ethyl]-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylate (**81**). In analogy to example **13** (route B), 1.9 g (95%) of the title compound **81** were obtained from 888 mg (3.98 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 1.1 g (4.38 mmol) of benzoate **80**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 (d, *J*=1.32 Hz, 1H), 8.24-8.33 (m, 2H), 7.92 (td, *J*=1.79, 8.48 Hz, 2H), 7.80 (dd, *J*=5.84, 8.48 Hz, 2H), 7.69 (d, *J*=8.29 Hz, 1H), 7.52 (t, *J*=7.50 Hz, 1H), 7.27 (t, *J*=7.16 Hz, 1H), 4.44-4.60 (m, 4H), 4.36 (q, *J*=7.16 Hz, 2H), 2.57-2.64 (m, 2H), 2.02 (s, 6H), 1.37 (t, *J*=7.06 Hz, 6H).

(c) 1-[2-(Dimethylamino)ethyl]-2-(9-ethyl-9*H*-carbazol-3-yl)-1*H*-benzimidazole-5-carboxylic acid (**15**). In analogy to example **13** (route B), 1.42 g (85%) of the title compound **15** were obtained from 1.7 g (3.74 mmol) of ethyl ester **81**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.73 (br s, 1H), 8.66 (d, *J*=1.52 Hz, 1H), 8.29 (d, *J*=7.53 Hz, 1H), 8.26 (s, 1H), 7.92 (td, *J*=1.58, 8.46 Hz, 2H), 7.81 (d, *J*=8.59 Hz, 1H), 7.76 (d, *J*=8.59 Hz, 1H), 7.69 (d, *J*=8.08 Hz, 1H), 7.52 (t, *J*=7.41 Hz, 1H), 7.27 (t, *J*=7.45 Hz, 1H), 4.46-4.58 (m, 4H), 2.59-2.68 (m, 2H), 2.03 (s, 6H), 1.38 (t, *J*=7.07 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 427.2134, found: 427.2141.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-phenyl-1*H*-benzimidazole-5-carboxylic acid (16). In analogy to example **13** (route A), 90 mg (22%) of the title compound **16** were obtained from 130 mg (0.58 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and 200 mg (0.88 mmol) of 3-amino-4-(phenylamino)benzoic acid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.79 (br s, 1H), 8.34-8.38 (m, 2H), 8.00 (d, *J*=7.58 Hz, 1H), 7.90 (dd, *J*=1.52, 8.59 Hz, 1H), 7.58-7.64 (m, 6H), 7.46-7.56 (m, 3H), 7.19-7.28 (m, 2H), 4.44 (q, *J*=7.07 Hz, 2H), 1.31 (t, *J*=1.00 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 432.1712, found: 432.1709.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylic acid (17). (a) Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate (**82**). In analogy to the synthesis of intermediate **60**, 761 mg (22%) of benzimidazole **82** were obtained from 3.0 g (8.12 mmol) of intermediate **59** and 2.29 mL (24.36 mmol) of 1-bromo-2-methoxyethane together with its regioisomer methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate (721 mg, 21%). Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate (**82**) ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.68 (d, *J*=1.32 Hz, 1H), 8.25 (s, 1H), 7.95-8.18 (m, 3H), 7.86 (d, *J*=8.48 Hz, 1H), 7.46-7.60 (m, 3H), 7.28-7.34 (m, 1H), 4.40-4.63 (m, 4H), 3.99 (s, 3H), 3.89 (t, *J*=5.65 Hz, 2H), 3.35 (s, 3H), 1.51 (t, *J*=7.16 Hz, 3H).

(b) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylic acid (**17**) In analogy to example **2**, 1.31 g of the title compound **17** were obtained by saponification of methyl ester **82** using 1N NaOH solution in ethanol/dichloromethane (10:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.80 (br s, 1H), 8.71 (d, *J*=1.32 Hz, 1H), 8.30 (s, 1H), 8.28 (d, *J*=7.66 Hz, 1H), 7.67-7.99 (m, 5H), 7.53 (t, *J*=7.49 Hz, 1H), 7.27 (t, *J*=7.16 Hz, 1H), 4.47-4.70 (m, 4H), 3.75

(t, *J*=5.09 Hz, 2H), 3.15 (s, 3H), 1.37 (t, *J*=6.97 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 414.1818, found: 414.1818.

Syntheses of compounds **18** – **25** can be found in the supporting information.

2-(9-Ethyl-9*H*-carbazol-3-yl)-4-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (26). (a) 3-(5-Bromo-4-fluoro-1*H*-benzimidazol-2-yl)-9-ethyl-9*H*-carbazole (**88**). In analogy to the preparation of intermediate **13** (route B), 6.5 g (69%) of the title compound **88** were obtained from 4.19 g (20.43 mmol) of 4-bromo-3-fluorobenzene-1,2-diamine and 4.56 g (20.43 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde. ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.33 (br s, 1H), 9.01 (br s, 1H), 8.18-8.39 (m, 2H), 7.81 (d, *J*=8.67 Hz, 1H), 7.68 (d, *J*=8.29 Hz, 1H), 7.26-7.56 (m, 4H), 4.51 (q, *J*=7.03 Hz, 2H), 1.36 (t, *J*=7.06 Hz, 3H).

(b) Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-4-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate (**90**). In analogy to the synthesis of intermediate **60**, 14.5 g (63%) of a mixture of 3-[5-bromo-4-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole **(89)** and its regiosisomer 3-[6-bromo-7-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole were obtained from 20 g (48.99 mmol) of intermediate **88** and 13.62 g (97.97 mmol) 1-bromo-2-methoxyethane. The regioisomers were not separated and used as a mixture in the following step.

Using an autoclave, the mixture of regioisomers (14.5 g, 31.09 mmol) was taken up in 70 mL of methanol, 5.08 g (6.22 mmol) of 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex and 12.21 g (124.37 mmol) of potassium acetate were added and the reaction vessel charged with carbon monoxide (35 bar) at RT. The reaction mixture was heated to 100 °C and kept at this temperature for 18 h. After cooling, the reaction mixture was

concentrated in vacuo and purified once by column chromatography (silica gel, hexane/ethyl acetate). The regiosomers were then separated by preparative HPLC to give 6.0 g (43%) of the title compound **90** together with 5.8 g (42%) of its regioisomer methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-7-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate. Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-4-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate (**90**) ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (d, *J*=1.27 Hz, 1H), 8.28 (d, *J*=7.65 Hz, 1H), 7.96 (dd, *J*=1.59, 8.58 Hz, 1H), 7.77-7.82 (m, 2H), 7.51-7.70 (m, 3H), 7.27 (t, *J*=7.31 Hz, 1H), 4.46-4.67 (m, 4H), 3.91 (s, 3H), 3.72 (t, *J*=5.25 Hz, 2H), 3.14 (s, 3H), 1.38 (t, *J*=7.15 Hz, 3H).

(c) 2-(9-Ethyl-9*H*-carbazol-3-yl)-4-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (**26**). 6.6 g (14.81 mmol) of methyl ester **90** was taken up in 150 mL of methanol/THF (2:1), treated with 37 mL of 2 M NaOH and stirred at 50 °C for 4 h. The mixture was acidified with 4M HCl, extracted with dichloromethane and the combined organic layers were dried down to give 6.3 g (96%) of the title compound **26**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.09-13.64 (m, 1H), 8.70 (d, *J*=1.26 Hz, 1H), 8.28 (d, *J*=7.58 Hz, 1H), 7.96 (dd, *J*=1.52, 8.59 Hz, 1H), 7.76-7.85 (m, 2H), 7.50-7.71 (m, 3H), 7.25-7.31 (m, 1H), 4.49-4.65 (m, 4H), 3.71 (t, *J*=5.31 Hz, 2H), 3.13 (s, 3H), 1.37 (t, *J*=7.07 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 432.1723, found: 432.1721.

4-Chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (27). (a) 3-(5-Bromo-4-chloro-1*H*-benzimidazol-2-yl)-9-ethyl-9*H*-carbazole (**91**). In analogy to the preparation of intermediate **13** (route B), 10.08 g of crude intermediate **91** (53% purity by LC-MS) were obtained from 4.2 g (18.96 mmol) of 4-bromo-3-clorobenzene-1,2-diamine and 4.23 g (18.96 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde and used in the next step without further purification.

(b) Methyl 4-chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate (**93**). In analogy to the synthesis of intermediate **60**, 2.47 g of a mixture of 3-[5-bromo-4-chloro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole (**92**) and its regiosisomer 3-[6-bromo-7-chloro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole were obtained from 2.8 g of crude intermediate **91** and 1.1 g (7.91 mmol) of 1-bromo-2-methoxyethane. The regioisomers were not separated and used as a mixture in the following step.

Using an autoclave, the mixture of regioisomers (1.2 g) was treated in analogy to intermediate **90** with 406 mg (0.497 mmol) of 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride dichloromethane complex and 975 mg (9.94 mmol) of potassium acetate, the reaction vessel was charged with carbon monoxide (14 bar) at RT and the mixture was heated to 100 °C overnight. After work up, the regiosomers were separated by preparative HPLC to give 250 mg (15%, 70% purity by LC-MS) of the title compound **93** together with 219 mg (17%, 89% purity by LCMS) of its regioisomer methyl 7-chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate. 100 mg of the title compound **93** were once again purified by preparative HPLC to finally give a pure sample (35 mg) of intermediate **93**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 (d, *J*=1.13 Hz, 1H), 8.30 (d, *J*=7.72 Hz, 1H), 7.96 (dd, *J*=1.60, 8.57 Hz, 1H), 7.77-7.86 (m, 3H), 7.70 (d, *J*=8.29 Hz, 1H), 7.54 (t, *J*=7.50 Hz, 1H), 7.27 (t, *J*=7.25 Hz, 1H), 4.47-4.68 (m, 4H), 3.90 (s, 3H), 3.70 (t, *J*=5.18 Hz, 2H), 3.12 (s, 3H), 1.37 (t, *J*=7.06 Hz, 3H).

(c) 4-Chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (**27**). In analogy to compound **26**, 92 mg of methyl ester **93** were saponified to give 85 mg (95%) of the title compound **27**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71-8.75 (m, 1H), 8.29 (d, *J*=7.58 Hz, 1H), 7.98 (dd, *J*=1.52, 8.59 Hz, 1H), 7.84-7.90 (m, 3H), 7.71 (d, *J*=8.34 Hz, 1H),

7.55 (t, *J*=7.33 Hz, 1H), 7.29 (t, *J*=7.45 Hz, 1H), 4.64 (br t, *J*=4.93 Hz, 2H), 4.55 (br d, *J*=7.07 Hz, 2H), 3.69-3.76 (m, 2H), 3.13 (s, 3H), 1.38 (t, *J*=7.07 Hz, 3H), COOH not stated; HRMS (ESI, $[M+H]^+$): calc.: 448.1428, found: 448.1423.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (28). (a) Methyl 4-[(2-methoxyethyl)amino]-2-methyl-3-nitrobenzoate (**53**). 27.5 g (0.12 mol) of a mixture of methyl 4-chloro-2-methyl-5-nitrobenzoate **(52)** and methyl 4-chloro-2-methyl-3-nitrobenzoate, prepared according to M. Baumgarth et al. , J. Med. Chem. 1997, 40, 2017-2034, were added to 50 mL of DMSO, treated with 31 mL (0.36 mol) of 2-methoxyethanamine and stirred at 80 °C for 25 h. The mixture was then treated with water, extracted several times with dichloromethane and the combined organic phases were evaporated. The residue was separated by chromatography on silica gel (hexane/dichloromethane) to give 9.3 g (29%) of methyl 4-[(2-methoxyethyl)amino]-2-methyl-3-nitrobenzoate and 15.5 g (49%) of methyl 4-[(2-methoxyethyl)amino]-2-methyl-5-nitrobenzoate. Methyl 4-[(2-methoxyethyl)amino]-2-methyl-3-nitrobenzoate **(53)**, 1H-NMR (400 MHz, DMSO-d_6), δ [ppm]= 7.83 (d, 1H), 6.86 (d, 1H), 6.42 (t, 1H), 3.77 (s, 3H), 3.47 (t, 2H), 3.36 (q, 2H), 3.26 (s, 3H),. 2.37 (s, 3H). Methyl 4-[(2-methoxyethyl)amino]-2-methyl-5-nitrobenzoate, ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (d, *J*=9.23 Hz, 1H), 6.85 (d, *J*=9.04 Hz, 1H), 6.44 (t, *J*=5.56 Hz, 1H), 3.76 (s, 3H), 3.46 (t, *J*=1.00 Hz, 2H), 3.33-3.39 (m, 2H), 3.25 (s, 3H), 2.36 (s, 3H).

(b) Methyl 3-amino-4-[(2-methoxyethyl)amino]-2-methylbenzoate (**54**). 3.33 g (12.4 mmol) of methyl 4-[(2-methoxyethyl)amino]-2-methyl-3-nitrobenzoate **(53)** were dissolved in 80 mL of THF/methanol (1:1) and hydrogenated under normal pressure on palladium (10% on charcoal). The catalyst was filtered off and the filtrate was concentrated. 2.85 g (92%) of crude methyl 3-amino-4-[(2-methoxyethyl)amino]-2-methylbenzoate **(54)** were thus obtained, which was used in

the following steps without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (d, *J*=8.59 Hz, 1H), 6.37 (d, *J*=8.59 Hz, 1H), 5.20 (t, *J*=5.31 Hz, 1H), 4.44 (br s, 2H), 3.70 (s, 3H), 3.53 (t, *J*=5.68 Hz, 2H), 3.25-3.30 (m, 5H), 2.31 (s, 3H).

(c) Methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**94**). In analogy to the preparation of intermediate **13** (route B), 1.7 g (84%) of the title compound **94** were obtained from 1.0 g (4.2 mmol) of intermediate **54** and 0.625 g (2.8 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (d, *J*=1.26 Hz, 1H), 8.28 (d, *J*=7.58 Hz, 1H), 7.93 (dd, *J*=1.64, 8.46 Hz, 1H), 7.84 (d, *J*=8.59 Hz, 1H), 7.80 (d, *J*=8.59 Hz, 1H), 7.69 (d, *J*=8.34 Hz, 1H), 7.60 (d, *J*=8.58 Hz, 1H), 7.52 (t, *J*=7.36 Hz, 1H), 7.26 (t, *J*=7.16 Hz, 1H), 4.49-4.59 (m, 4H), 3.87 (s, 3H), 3.26-3.30 (m, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 1.38 (t, *J*=7.07 Hz, 3H).

(d) 2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**28**). In analogy to compound **26**, 1.13 g of methyl ester **94** were saponified to give 691 mg (63%) of the title compound **28**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.61 (br s, 1H), 8.65 (d, *J*=1.32 Hz, 1H), 8.28 (d, *J*=7.72 Hz, 1H), 7.77-7.95 (m, 3H), 7.69 (d, *J*=8.10 Hz, 1H), 7.48-7.61 (m, 2H), 7.26 (t, *J*=7.35 Hz, 1H), 4.45-4.63 (m, 4H), 3.61-3.74 (m, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 1.37 (t, *J*=7.06 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 428.1974, found: 428.1973.

2-(9-Ethyl-9*H*-carbazol-3-yl)-6-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (29). (a) 3-(5-Bromo-6-fluoro-1*H*-benzimidazol-2-yl)-9-ethyl-9*H*-carbazole (**95**). In analogy to the preparation of intermediate **13** (route B), 4.6 g (58%) of the title compound **95** were obtained from 4.0 g (19.51 mmol) of 4-bromo-5-fluorobenzene-1,2-diamine and 4.36 g (19.51 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde. ^{1}H NMR (400 MHz, CHLOROFORM-d)

δ 8.75 (d, *J*=1.51 Hz, 1H), 8.12 (d, *J*=7.56 Hz, 1H), 8.09 (d, *J*=8.57 Hz, 1H), 7.79 (br s, 1H), 7.38-7.55 (m, 4H), 7.27-7.31 (m, 1H), 4.40 (q, *J*=7.28 Hz, 2H), 1.47 (t, *J*=7.28 Hz, 3H), NH not stated.

(b) 2-(9-Ethyl-9*H*-carbazol-3-yl)-6-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (**29**). In analogy to the synthesis of intermediate **60**, 1.83 g (69%) of a mixture of 3-[5-bromo-6-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole **(96)** and its regiosisomer 3-[6-bromo-5-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole were obtained from 2.25 g (5.51 mmol) of intermediate **95** and 1.53 g (11.02 mmol) of 1-bromo-2-methoxyethane. The regioisomers were not separated and used as a mixture in the following step.

The mixture of regioisomers (1.8 g, 3.86 mmol) was dissolved in 36 mL of THF, 1.4 mL of methanol, 1.53 g (5.79 mmol) of molybdenehexacarbonyl, 1.73 mL of 1,8-diazabicyclo[5.4.0]undec-7-ene **(**DBU, 11.58 mmol), 292 mg (0.39 mmol) of trans-bis-(acetato)-bis-[o-(di-o-tolylphosphino)-benzyl]-dipalladium and 112 mg (0.39 mmol) of tri-tert-butylphosphonium tetrafluoroborate were added and the reaction mixture was heated to 100 °C for 25 min. using a microwave (100W, 6 bar). The reaction mixture was cooled down, extracted with dichloromethane/water and the combined organic layers were concentrated in vacuo to give 3.14 g of a crude mixture of methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-6-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate **(97)** and its regioisomer methyl 2-(9-ethyl-9*H*-carbazol-3-yl)-5-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate. The regioisomers were not separated and used as a mixture in the final step.

In analogy to compound **26**, 3.14 g of a mixture of regioisomers obtained in the preceding step were saponified to again give a mixture of the two corresponding carboxylic acids. The two regioisomers were separated by preparative HPLC to give 93 mg (0.2 mmol) of the title compound **29** alongside 109 mg (0.25 mmol) of its regioisomer 2-(9-ethyl-9*H*-carbazol-3-yl)-5-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylic acid. 2-(9-Ethyl-9*H*-carbazol-3-yl)-6-fluoro-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid **(29)**, ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.40-13.67 (m, 1H), 8.66 (d, *J*=1.51 Hz, 1H), 8.23-8.30 (m, 1H), 8.15 (d, *J*=6.78 Hz, 1H), 7.93 (dd, *J*=1.76, 8.53 Hz, 1H), 7.79 (d, *J*=8.53 Hz, 1H), 7.69-7.71 (m, 1H), 7.67 (s, 1H), 7.53 (t, *J*=7.72 Hz, 1H), 7.27 (t, *J*=7.15 Hz, 1H), 4.48-4.62 (m, 4H), 3.69 (t, *J*=5.14 Hz, 2H), 3.14 (s, 3H), 1.38 (t, *J*=7.15 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 432.1723, found: 432.1723.

2-(9-Ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-6-methyl-1*H*-benzimidazole-5-carboxylic acid (30). 160 mg (0.816 mmol) of 4-amino-2-methyl-5-nitrobenzoic acid in 3.15 mL of DMF were treated with 39 mg (0.897 mmol, 55%) of NaH and stirred at RT for 20 min. 125 mg (0.897 mmol) of 1-bromo-2-methoxyethane were added and the mixture was stirred at 80 °C for 3 h. After cooling to RT another 39 mg of NaH and 125 mg of 1-bromo-2-methoxyethane were added and stirring continued for additional 3 hours at 80 °C. The solvents were then removed under reduced pressure to give 207 mg of 4-[(2-methoxyethyl)amino]-2-methyl-5-nitrobenzoic acid as a crude mixture also containing some double alkylation product as major impurity. This mixture was used in the subsequent steps without further purification.

The crude material (207 mg) obtained in the previous step was dissolved in 12 mL of methanol and hydrogenated under normal pressure on palladium (10% on charcoal) over night. The catalyst was filtered off and the filtrate was concentrated to give 504 mg of 5-amino-4-[(2-

methoxyethyl)amino]-2-methylbenzoic acid as a crude mixture also containing some double alkylation product as major impurity.

In analogy to example **13** (route A), 500 mg of crude 5-amino-4-[(2-methoxyethyl)amino]-2-methylbenzoic acid and 453 mg (2.03 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde were converted to crude compound **30**. This material was again taken up in ethanol/water (1:1), treated with 2N NaOH and heated to100 °C for 30 min. using a microwave. After cooling, the reaction mixture was acidified with 1N HCl and dried down. After preparative HPLC 11.5 mg of the title compound **30** were finally obtained. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.58 (br s, 1H), 8.68 (s, 1H), 8.27 (d, *J*=7.57 Hz, 1H), 8.20 (s, 1H), 7.94 (d, *J*=8.33 Hz, 1H), 7.80 (d, *J*=8.67 Hz, 1H), 7.69 (d, *J*=8.10 Hz, 1H), 7.61 (s, 1H), 7.52 (t, *J*=7.72 Hz, 1H), 7.27 (t, *J*=7.25 Hz, 1H), 4.47-4.59 (m, 4H), 3.72 (t, *J*=5.27 Hz, 2H), 3.14 (s, 3H), 2.70 (s, 3H), 1.37 (t, *J*=6.97 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 428.1974, found: 428.1969.

6-Chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (31). (a) 3-(5-Bromo-6-chloro-1*H*-benzimidazol-2-yl)-9-ethyl-9*H*-carbazole (**98**). In analogy to the preparation of intermediate **13** (route B), 3.3 g of intermediate **98** (97%) were obtained from 1.6 g (7.22 mmol) of 4-bromo-5-chlorobenzene-1,2-diamine and 1.07 g (4.82 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (br d, *J*=4.80 Hz, 1H), 8.98 (d, *J*=1.26 Hz, 1H), 8.22-8.32 (m, 2H), 7.98-8.20 (m, 1H), 7.89 (d, *J*=17.18 Hz, 1H), 7.75-7.83 (m, 1H), 7.68 (d, *J*=8.34 Hz, 1H), 7.49-7.59 (m, 1H), 7.26-7.32 (m, 1H), 4.51 (q, *J*=7.07 Hz, 1H), 4.45-4.59 (m, 1H), 1.35 (t, *J*=7.07 Hz, 3H).

(b) 6-Chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylic acid (**31**). In analogy to the synthesis of intermediate **60**, 1.55 g of a mixture of 3-[5-bromo-6-

chloro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole **(99)** and its regiosisomer 3-[6-bromo-5-chloro-1-(2-methoxyethyl)-1*H*-benzimidazol-2-yl]-9-ethyl-9*H*-carbazole were obtained from 3.3 g of intermediate **98** and 2.59 g (18.65 mmol) of 1-bromo-2-methoxyethane The regioisomers were not separated and used as a mixture in the following step.

In analogy to the methoxycarbonylation of bromide **96**, 100 mg of bromide **99** were treated with 8.1 mg (0.01 mmol) of trans-bis-(acetato)-bis-(0-(di-o-tolyl)-phosphino)-benzyl)-dipalladium, 41 mg (0.155 mmol) of molybdenehexacarbonyl, 0.046 mL of DBU (0.311 mmol) and 3.1 mg (0.01 mmol) of tri-tert-butylphosphonium tetrafluoroborate to give 10 mg of a mixture of methyl 6-chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-5-carboxylate **(100)** and its regioisomer methyl 5-chloro-2-(9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-1*H*-benzimidazole-6-carboxylate. The regioisomers were not separated and used as a mixture in the final saponification step.

In analogy to compound **26**, 15 mg of a mixture of regioisomers obtained in the previous step were saponified to give after preparative HPLC 3.0 mg of the title compound **31**. ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.99-13.98 (m, 1H), 8.67 (d, *J*=1.51 Hz, 1H), 8.27 (d, *J*=7.53 Hz, 1H), 8.04 (s, 1H), 7.90-7.97 (m, 1H), 7.84 (s, 1H), 7.79 (d, *J*=8.66 Hz, 1H), 7.65-7.72 (m, 1H), 7.49-7.56 (m, 1H), 7.25-7.29 (m, 1H), 4.50-4.60 (m, 4H), 3.69 (t, *J*=5.27 Hz, 2H), 3.14 (s, 3H), 1.38 (t, *J*=7.15 Hz, 3H).

2-(9-Ethyl-6-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (32). (a) 6-Bromo-9-ethyl-9*H*-carbazole-3-carbaldehyde (**56**). 15 g (67.18 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde were dissolved in 230 mL of acetonitrile, treated with 13.15 g (73.9 mmol) of N-bromosuccinimide (NBS) and stirred for 1 h at RT. The

precipitate formed was filtered off and washed with little methanol to give 15.13 g (75%) of the title compound **56**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.83 (d, *J*=1.13 Hz, 1H), 8.56 (d, *J*=1.32 Hz, 1H), 8.03 (dd, *J*=1.60, 8.57 Hz, 1H), 7.81 (d, *J*=8.67 Hz, 1H), 7.64-7.73 (m, 2H), 4.51 (q, *J*=7.03 Hz, 2H), 1.33 (t, *J*=7.16 Hz, 3H).

(b) 9-Ethyl-6-methyl-9*H*-carbazole-3-carbaldehyde (**57**). 15 g (49.64 mmol) of bromide **56** and 4.457 g (74.46 mmol) of methylboronic acid were dissolved in 150 mL of 1,4-dioxane, treated with 5.736 g (4.964 mmol) of tetrakis(triphenylphosphine)palladium(0) and 48.52 g (148.92 mmol) of cesium carbonate and stirred at 100 °C until complete conversion of the starting material. The solids were filtered off, washed with ethyl acetate and the combined organic layers were dried down. The residue was purified by column chromatography (silica gel, hexane/ethyl acetate) to give 10.5 g (71%) of the title compound **57**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 8.70 (d, *J*=1.01 Hz, 1H), 8.07-8.09 (m, 1H), 7.95-8.01 (m, 1H), 7.67-7.78 (m, 1H), 7.59 (d, *J*=8.34 Hz, 1H), 7.33-7.39 (m, 1H), 4.44-4.54 (m, 2H), 1.33 (t, *J*=1.00 Hz, 3H), CH_3 not stated.

(c) Methyl 2-(9-ethyl-6-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**58**). In analogy to the preparation of intermediate **13** (route B), 3.4 g intermediate **58** (74%) were obtained from 2.41 g (10.11 mmol) of intermediate **54** and 2.4 g (10.11 mmol) of intermediate **57**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (d, *J*=1.26 Hz, 1H), 8.06 (s, 1H), 7.90 (d, *J*=8.58 Hz, 1H), 7.83 (d, *J*=8.24 Hz, 1H), 7.75 (d, *J*=8.59 Hz, 1H), 7.58 (t, *J*=8.84 Hz, 2H), 7.35 (dd, *J*=1.01, 8.34 Hz, 1H), 4.45-4.60 (m, 4H), 3.87 (s, 3H), 3.69 (t, *J*=5.31 Hz, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 2.49 (s, 3H), 1.35 (t, *J*=7.07 Hz, 3H).

(d) 2-(9-Ethyl-6-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**32**). In analogy to example **2**, 2.9 g (88%) of the title compound **32** were obtained by saponification of 3.4 g (7.463 mmol) of methyl ester **58** using 2N NaOH in methanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (br s, 1H), 8.59 (d, *J*=1.52 Hz, 1H), 8.06 (s, 1H), 7.89 (d, *J*=8.06 Hz, 1H), 7.84 (d, *J*=8.19 Hz, 1H), 7.74 (d, *J*=8.59 Hz, 1H), 7.56 (dd, *J*=4.42, 8.46 Hz, 2H), 7.34 (d, *J*=8.40 Hz, 1H), 4.44-4.59 (m, 4H), 3.69 (t, *J*=5.31 Hz, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 2.49 (s, 3H), 1.35 (t, *J*=7.20 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 442.2131, found: 442.2129.

2-(6-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (33). (a) 6-Chloro-9-ethyl-9*H*-carbazole-3-carbaldehyde (91) and 8-chloro-9-ethyl-9*H*-carbazole-3-carbaldehyde (**102**). 2 g (8.96 mmol) of 9-ethyl-9*H*-carbazole-3-carbaldehyde were dissolved in 40 mL of acetonitrile, treated with 1.316 g (9.85 mmol) of N-chlorosuccinimide (NCS) and stirred for 2.5 hours at 40 °C. The mixture was cooled down and kept overnight at RT. The reaction mixture was dried down and the residue purified by column chromatography (silica gel, hexane/ethyl acetate) to give 1.65 g (68%) of the title compound **101** and 0.47 g (20%) of the regioisomer 8-chloro-9-ethyl-9*H*-carbazole-3-carbaldehyde (**102**). 6-Chloro-9-ethyl-9*H*-carbazole-3-carbaldehyde (**101**), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.82 (d, *J*=1.01 Hz, 1H), 8.43 (d, *J*=1.77 Hz, 1H), 8.03 (dd, *J*=1.52, 8.59 Hz, 1H), 7.81 (d, *J*=8.31 Hz, 1H), 7.76 (d, *J*=8.74 Hz, 1H), 7.54-7.58 (m, 1H), 4.52 (q, *J*=7.07 Hz, 2H), 1.33 (t, *J*=7.20 Hz, 3H). 8-Chloro-9-ethyl-9*H*-carbazole-3-carbaldehyde (**102**), ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H), 8.82 (d, *J*=1.26 Hz, 1H), 8.43 (d, *J*=2.02 Hz, 1H), 8.03 (dd, *J*=1.77, 8.59 Hz, 1H), 7.81 (d, *J*=8.34 Hz, 1H), 7.75 (d, *J*=8.76 Hz, 1H), 7.56 (dd, *J*=2.15, 8.72 Hz, 1H), 4.52 (q, *J*=7.16 Hz, 2H), 1.33 (t, *J*=7.20 Hz, 3H).

(b) Methyl 2-(6-chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**103**). In analogy to the preparation of intermediate **13** (route B), 1.32 g of intermediate **103** (93%) were obtained from 707 mg (2.967 mmol) of intermediate **54** and 765 mg (2.967 mmol) of intermediate **101**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (d, *J*=1.52 Hz, 1H), 8.41 (d, *J*=2.02 Hz, 1H), 7.96 (d, *J*=8.68 Hz, 1H), 7.83 (t, *J*=7.33 Hz, 2H), 7.73 (d, *J*=8.59 Hz, 1H), 7.60 (d, *J*=8.04 Hz, 1H), 7.53 (d, *J*=8.79 Hz, 1H), 4.45-4.66 (m, 4H), 3.87 (s, 3H), 3.62-3.73 (m, 2H), 3.09 (s, 3H), 2.89 (s, 3H), 1.36 (t, *J*=7.07 Hz, 3H).

(c) 2-(6-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**33**). In analogy to example **2**, 820 mg (99%) of the title compound **33** were obtained by saponification of 850 mg (1.786 mmol) of methyl ester **103** using 2N NaOH in methanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.65 (br s, 1H), 8.71 (s, 1H), 8.41 (d, *J*=2.02 Hz, 1H), 7.98 (dd, *J*=1.52, 8.59 Hz, 1H), 7.88 (br d, *J*=8.59 Hz, 1H), 7.85 (br d, *J*=8.59 Hz, 1H), 7.74 (d, *J*=8.84 Hz, 1H), 7.63 (d, *J*=8.59 Hz, 1H), 7.54 (dd, *J*=2.15, 8.72 Hz, 1H), 4.49-4.63 (m, 4H), 3.68 (t, *J*=5.18 Hz, 2H), 3.10 (s, 3H), 2.89 (s, 3H), 1.36 (t, *J*=7.07 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 462.1584, found: 462.1578.

2-[9-Ethyl-6-(trifluoromethyl)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1H-benzimidazole-5-carboxylic acid (34). (a) Methyl 2'-amino-6-fluoro-5'-(trifluoromethyl)[biphenyl]-3-carboxylate (**104**). 2.961 g (10.57 mmol) of methyl 4-fluoro-3-iodobenzoate, 1.97 g (9.61 mmol) of [2-amino-5-(trifluoromethyl)phenyl]boronic acid, 1.015 g (1.44 mmol) of bis(triphenylphosphine)palladium(II) dichloride and 454 mg (1.73 mmol) of triphenylphosphine were taken up in 96 mL of THF and treated with 23 mL of 1 M aqueous potassium carbonate solution. The reaction mixture was heated to 65 °C for 5 hours, then the THF was evaporated and the remaining mixture was extracted with ethyl acetate. The organic

layer was dried down and the residue purified by column chromatography (silica gel, hexane/ethyl acetate) to give 3.98 g (54%) of the title compound **104**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.04 (ddd, *J*=2.27, 5.05, 8.59 Hz, 1H), 7.91 (dd, *J*=2.27, 7.07 Hz, 1H), 7.46 (t, *J*=8.94 Hz, 1H), 7.41 (d, *J*=8.64 Hz, 1H), 7.25 (s, 1H), 6.87 (d, *J*=8.59 Hz, 1H), 5.61 (br s, 2H), 3.86 (s, 3H).

(b) 6-(Trifluoromethyl)-9*H*-carbazole-3-carboxylic acid (**105**). 2.8 g (8.94 mmol) of intermediate **104** were dissolved in 31 mL of DMF and treated with 1.138 g of NaH (60%, 28.44 mmol). The reaction mixture was heated to 90 °C for 23 h, cooled down to RT and carefully treated with water. The aqueous phase was acidified with 2N HCl (pH 3) and extracted with ethyl acetate. The organic layer was dried down and the residue was purified by column chromatography (silica gel, hexane/ethyl acetate) to give 500 mg (20%) of the title compound **105**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1H), 8.96 (s, 1H), 8.74 (s, 1H), 8.07 (dd, *J*=1.52, 8.59 Hz, 1H), 7.70-7.76 (m, 2H), 7.57-7.66 (m, 2H).

(c) Ethyl 9-ethyl-6-(trifluoromethyl)-9*H*-carbazole-3-carboxylate (**106**). 500 mg (1.79 mmol) of intermediate **105** were dissolved in 27 mL of acetone and treated with 1.0 mL (12.53 mmol) of ethyl iodide as well as 1.75 g of cesium carbonate (5.37 mmol). The reaction mixture was stirred at RT for 3 h and the solvent removed. The residue was taken up in ethyl acetate and washed with water. The organic layer was dried down to give 750 mg of crude intermediate **106** which was used in the saponification step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.02 (d, *J*=1.27 Hz, 1H), 8.83 (s, 1H), 8.14 (d, *J*=8.62 Hz, 1H), 7.74-7.92 (m, 3H), 4.56 (q, *J*=7.27 Hz, 2H), 4.32-4.40 (m, 2H), 1.31-1.41 (m, 6H).

(d) 9-Ethyl-6-(trifluoromethyl)-9*H*-carbazole-3-carboxylic acid (**107**). 750 mg of crude ethyl ester **106** were saponified using 1.23 mL of 2N sodium hydroxide (2.46 mmol) in ethanol/THF at 80 °C. The reaction mixture was extracted with ethyl acetate, the aqueous phase adjusted to pH 3 with 2N HCl and extracted several times with ethyl acetate. The combined organic layers were evaporated to dryness to give 180 mg (0.56 mmol) of the title compound **107**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.62 (br s, 1H), 8.99 (d, *J*=1.26 Hz, 1H), 8.79 (s, 1H), 8.10-8.17 (m, 1H), 7.75-7.91 (m, 3H), 4.55 (q, *J*=7.07 Hz, 2H), 1.35 (t, *J*=7.07 Hz, 3H).

(e) 9-Ethyl-6-(trifluoromethyl)-9*H*-carbazole-3-carbonyl chloride (**108**). 180 mg of carboxylic acid **107** were taken up in 4.7 mL of toluene and treated with 0.51 mL (7.03 mmol) of thionyl chloride. The mixture was heated to 110 °C for 3 h, cooled to RT and dried down to give 252 mg of crude acid chloride **108** which was immediately used in the next step.

(f) Methyl 2-[9-ethyl-6-(trifluoromethyl)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**109**). 161 mg (0.675 mmol) of intermediate **54** and 220 mg (0.675 mmol) of intermediate **108** in 8 mL of acetonitrile were heated in a microwave at 150 °C for 40 minutes. After cooling to RT, the solvent was evaporated and the residue purified by preparative HPLC to give 170 mg (47%) of the title compound **109**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, *J*=1.26 Hz, 1H), 8.77 (s, 1H), 8.02 (dd, *J*=1.52, 8.59 Hz, 1H), 7.80-7.93 (m, 4H), 7.61 (d, *J*=8.59 Hz, 1H), 4.53-4.65 (m, 4H), 3.87 (s, 3H), 3.66 (t, *J*=5.31 Hz, 2H), 3.08 (s, 3H), 2.89 (s, 3H), 1.39 (t, *J*=7.07 Hz, 3H).

(g) 2-[9-Ethyl-6-(trifluoromethyl)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**34**). In analogy to example **2**, 30 mg (53%) of the title compound **34** were obtained by saponification of 58 mg (0.113 mmol) of methyl ester **109** using

2N NaOH in THF/methanol (1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34-13.23 (m, 1H), 8.88 (s, 1H), 8.77 (s, 1H), 8.05 (dd, *J*=1.27, 8.62 Hz, 1H), 7.83-7.98 (m, 4H), 7.69 (br d, *J*=8.36 Hz, 1H), 4.55-4.70 (m, 4H), 3.68 (br t, *J*=5.20 Hz, 2H), 3.09 (s, 3H), 2.90 (s, 3H), 1.39 (t, *J*=7.10 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 496.1848, found: 496.1849.

2-[9-Ethyl-6-(trifluoromethoxy)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (35). (a) Ethyl 2'-amino-6-fluoro-5'-(trifluoromethoxy) [biphenyl]-3-carboxylate (**110**). In analogy to intermediate **104**, 5.87 g (51%) of intermediate **110** were obtained from 8 g (37.74 mmol) of [5-(ethoxycarbonyl)-2-fluorophenyl]boronic acid and 6.44 g (25.16 mmol) of 2-bromo-4-(trifluoromethoxy)aniline. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.02 (ddd, *J*=2.35, 4.99, 8.67 Hz, 1H), 7.91 (dd, *J*=2.26, 7.16 Hz, 1H), 7.39-7.50 (m, 1H), 7.12 (dd, *J*=2.07, 8.85 Hz, 1H), 6.98 (d, *J*=2.64 Hz, 1H), 6.81 (d, *J*=8.85 Hz, 1H), 4.24-4.39 (m, 2H), 1.28-1.39 (m, 3H), NH2 not stated.

(b) 6-(Trifluoromethoxy)-9*H*-carbazole-3-carboxylic acid (**111**). In analogy to intermediate **105**, 5.87 g (17.1 mmol) of intermediate **110** were treated with 2.39 g of NaH (60%, 59,85 mmol) to give 14.58 g of crude intermediate **111** which was used without purification in the next step.

(c) Ethyl 9-ethyl-6-(trifluoromethoxy)-9*H*-carbazole-3-carboxylate (**112**). In analogy to intermediate **106**, 14.58 g of crude intermediate **111** were treated with 48 mL of ethyl iodide (0.315 mol) and 73.47 g of cesium carbonate (0.225 mol) to give 1.85 g of crude intermediate **112** which was used in the next step.

(d) 9-Ethyl-6-(trifluoromethoxy)-9*H*-carbazole-3-carboxylic acid (**113**). 1.85 g of crude ethyl ester **112** were saponified in analogy to intermediate **107** using 2N NaOH to give 1.41g (4.36 mmol, 25% over 3 steps) of the title compound **113**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05-

13.38 (m, 1H), 8.92 (d, *J*=1.25 Hz, 1H), 8.40 (s, 1H), 8.10 (dd, *J*=1.63, 8.66 Hz, 1H), 7.68-7.82 (m, 2H), 7.49 (dd, *J*=1.51, 8.78 Hz, 1H), 4.52 (q, *J*=7.19 Hz, 1H), 4.46-4.59 (m, 1H), 1.34 (t, *J*=1.00 Hz, 3H).

(e) 9-Ethyl-6-(trifluoromethoxy)-9*H*-carbazole-3-carbonyl chloride (**114**). In analogy to intermediate **108**, 150 mg (0.464 mmol) of intermediate **113** were treated with 0.41 mL of thionyl chloride (5.57 mmol) to give 120 mg of crude intermediate **114** which was immediately used in the next step.

(f) Methyl 2-[9-ethyl-6-(trifluoromethoxy)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**115**). In analogy to intermediate **109**, 60 mg (0.252 mmol) of intermediate **54** and 86 mg (0.252 mmol) of intermediate **114** gave 40 mg (30%) of the title compound **115**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.73-8.77 (m, 1H), 8.38 (s, 1H), 7.99 (dd, *J*=1.51, 8.48 Hz, 1H), 7.78-7.89 (m, 3H), 7.61 (d, *J*=8.67 Hz, 1H), 7.51 (br d, *J*=8.10 Hz, 1H), 4.49-4.64 (m, 4H), 3.87 (s, 3H), 3.66 (t, *J*=5.27 Hz, 2H), 3.09 (s, 3H), 2.89 (s, 3H), 1.38 (t, *J*=6.97 Hz, 3H).

(g) 2-[9-Ethyl-6-(trifluoromethoxy)-9*H*-carbazol-3-yl]-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**35**). In analogy to example **34**, 20 mg (61%) of the title compound **35** were obtained by saponification of 34 mg (0.064 mmol) of methyl ester **115**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (br s, 1H), 8.74 (d, *J*=1.27 Hz, 1H), 8.39 (d, *J*=1.27 Hz, 1H), 7.98 (dd, *J*=1.77, 8.62 Hz, 1H), 7.85 (d, *J*=8.87 Hz, 2H), 7.80 (d, *J*=9.12 Hz, 1H), 7.57 (d, *J*=8.05 Hz, 1H), 7.51 (d, *J*=8.94 Hz, 1H), 4.49-4.63 (m, 4H), 3.66 (t, *J*=5.32 Hz, 2H), 3.09 (s, 3H), 2.89 (s, 3H), 1.38 (t, *J*=7.10 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 512.1797, found: 512.1797.

2-(5-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (36). (a) Ethyl 2'-amino-6'-chloro-6-fluoro[biphenyl]-3-carboxylate (**116**). In analogy to intermediate **104**, 500 mg (19%) of intermediate **116**were obtained from 1.90 g (8.98 mmol) of [5-(ethoxycarbonyl)-2-fluorophenyl]boronic acid and 2.27 g (8.98 mmol) of 3-chloro-2-iodoaniline. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.06 (ddd, *J*=2.26, 5.09, 8.67 Hz, 1H), 7.79 (dd, *J*=2.26, 6.97 Hz, 1H), 7.47 (t, *J*=8.95 Hz, 1H), 7.10 (t, *J*=8.01 Hz, 1H), 6.71 (dd, *J*=3.96, 8.10 Hz, 2H), 5.04 (s, 2H), 4.31 (q, *J*=7.16 Hz, 2H), 1.32 (t, *J*=7.06 Hz, 3H).

(b) 5-Chloro-9*H*-carbazole-3-carboxylic acid (**117**). In analogy to intermediate **105**, 1.1 g (3.74 mmol) of intermediate **116** were treated with 315 mg of NaH (60%) to give 500 mg crude intermediate **117** which was used without further purification in the next step.

(c) Ethyl 5-chloro-9-ethyl-9*H*-carbazole-3-carboxylate (**118**). In analogy to intermediate **106**, 500 mg crude intermediate **117** were treated with 0.81 mL of ethyl iodide (10.18 mmol) and 1.99 g of cesium carbonate (6.11 mmol) to give 400 mg of crude intermediate **118** which was used in the next step.

(d) 5-Chloro-9-ethyl-9*H*-carbazole-3-carboxylic acid (**119**). 400 mg crude of ethyl ester **118** were saponified in analogy to intermediate **107** using 3.31 mL of 2N NaOH to give 110 mg (0.4 mmol, 11% over 3 steps) of the title compound **119**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.38-13.19 (m, 1H), 9.10 (d, *J*=1.32 Hz, 1H), 8.14 (dd, *J*=1.51, 8.67 Hz, 1H), 7.64-7.83 (m, 2H), 7.53 (t, *J*=8.01 Hz, 1H), 7.33 (d, *J*=7.72 Hz, 1H), 4.53 (q, *J*=7.22 Hz, 2H), 1.33 (t, *J*=7.06 Hz, 3H).

(e) Methyl 2-(5-chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**120**). 45 mg (0.189 mmol) of intermediate **54** and 52 mg (0.189 mmol) of intermediate **119** in 1.46 mL of ethyl acetate were treated with 50 mg of powdered

molecular sieve, 0.38 mL of pyridine (4.72 mmol) and 1.12 mL (1.89 mmol) of propylphosphonic anhydride (50% in DMF). This mixture was heated in a microwave at 120 °C (5 bar) for 45 minutes. The mixture was cooled down, taken up with water, extracted with ethyl acetate and the combined organic layers were evaporated to give 15 mg (17%) of the title compound **120** after column chromatography (silica gel, hexane/ethyl acetate).

(f) 2-(5-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**36**). In analogy to example **34**, 10 mg (69%) of the title compound **36** were obtained by saponification of 15 mg (0.032 mmol) of methyl ester **120**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.58 (br s, 1H), 8.95 (d, *J*=1.26 Hz, 1H), 8.03 (dd, *J*=1.77, 8.59 Hz, 1H), 7.89 (d, *J*=8.16 Hz, 1H), 7.85 (d, *J*=8.21 Hz, 1H), 7.73 (d, *J*=7.83 Hz, 1H), 7.51-7.59 (m, 2H), 7.33 (d, *J*=7.58 Hz, 1H), 4.48-4.62 (m, 4H), 3.75 (t, *J*=5.43 Hz, 2H), 3.15 (s, 3H), 2.89 (s, 3H), 1.38 (t, *J*=7.07 Hz, 3H).

2-(9-Ethyl-5-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (37). (a) Ethyl 2'-amino-6-fluoro-6'-methyl[biphenyl]-3-carboxylate (**121**). In analogy to intermediate **104**, 1.2 g (51%) of intermediate **121** were obtained from 2.73 g (12.87 mmol) of [5-(ethoxycarbonyl)-2-fluorophenyl]boronic acid and 2 g (8.58 mmol) of 2-iodo-3-methylaniline. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94-8.06 (m, 1H), 7.65-7.79 (m, 1H), 7.39-7.49 (m, 1H), 7.00 (t, *J*=7.72 Hz, 1H), 6.80-6.91 (m, 1H), 6.61 (d, *J*=8.10 Hz, 1H), 4.42-4.92 (m, 2H), 4.31 (q, *J*=7.03 Hz, 2H), 1.86 (s, 3H), 1.31 (t, *J*=1.00 Hz, 3H).

(b) 5-Methyl-9*H*-carbazole-3-carboxylic acid (**122**). In analogy to intermediate **105**, 2.7 g (9.88 mmol) of intermediate **121** were treated with 1.14 g of NaH (60%) to give 640 mg of crude intermediate **122** which was used without purification in the next step.

(c) Ethyl 9-ethyl-5-methyl-9*H*-carbazole-3-carboxylate (**123**). In analogy to intermediate **106**, 640 mg of crude intermediate **122** were treated with 1.71 mL of ethyl iodide (21.31 mmol) and 4.63 g of cesium carbonate (14.21 mmol) to give 1.16 g of crude intermediate **123** which was used without further purification in the next step.

(d) 9-Ethyl-5-methyl-9*H*-carbazole-3-carboxylic acid (**124**). 1.16 g crude of ethyl ester **123** were saponified in analogy to intermediate **107** using 10 mL of 2N NaOH to give 1.03 g (3.78 mmol, 38% over 3 steps) of the title compound **124**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.68 (br s, 1H), 8.74 (d, *J*=1.32 Hz, 1H), 8.07 (dd, *J*=1.60, 8.57 Hz, 1H), 7.69 (d, *J*=8.67 Hz, 1H), 7.52 (d, *J*=8.29 Hz, 1H), 7.41 (t, *J*=7.63 Hz, 1H), 7.07 (d, *J*=7.35 Hz, 1H), 4.48 (q, *J*=6.97 Hz, 2H), 2.84 (s, 3H), 1.26-1.39 (m, 3H).

(e) Methyl 2-(9-ethyl-5-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**125**). In analogy to intermediate **120**, 200 mg (0.84 mmol) of intermediate **54** and 213 mg (0.84 mmol) of intermediate **124** gave 250 mg of crude material of the title compound **125**.

(f) 2-(9-Ethyl-5-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**37**). In analogy to example **34**, 11 mg (5%) of the title compound **37** were obtained by saponification of 250 mg (0.548 mmol) of methyl ester **125** after purification by preparative HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.51 (br s, 1H), 8.68 (s, 1H), 7.94 (dd, *J*=1.52, 8.59 Hz, 1H), 7.83 (t, *J*=10.02 Hz, 2H), 7.50-7.59 (m, 2H), 7.43 (t, *J*=7.71 Hz, 1H), 7.07 (d, *J*=7.07 Hz, 1H), 4.47-4.60 (m, 4H), 3.80 (t, *J*=5.18 Hz, 2H), 3.19 (s, 3H), 2.90 (s, 3H), 2.87 (s, 3H), 1.36 (t, *J*=7.07 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 442.2131, found: 442.2125.

2-(8-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (38). (a) Methyl 2-(8-chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**126**). In analogy to the preparation of intermediate **13** (route B), 2.12 g of intermediate **126** (99%) were obtained from 1.03 g (4.32 mmol) of intermediate **54** and 742 mg (2.88 mmol) of intermediate **102** which was used in the final step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (d, *J*=1.52 Hz, 1H), 8.24-8.34 (m, 1H), 7.95-8.03 (m, 1H), 7.82-7.90 (m, 2H), 7.53-7.66 (m, 2H), 7.24-7.32 (m, 1H), 4.83-4.92 (m, 2H), 4.55 (t, *J*=5.18 Hz, 2H), 3.87 (s, 3H), 3.65-3.73 (m, 2H), 3.11 (s, 3H), 2.89 (s, 3H), 1.43 (t, *J*=7.07 Hz, 3H).

(b) 2-(8-Chloro-9-ethyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**38**). In analogy to example **2**, 700 mg (1.47 mmol, 51% over 2 steps) of the title compound **38** were obtained by saponification of 2.12 g of methyl ester **126** using 2N NaOH in methanol. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.56 (br s, 1H), 8.69 (d, *J*=1.32 Hz, 1H), 8.30 (dd, *J*=0.94, 7.72 Hz, 1H), 7.93-8.04 (m, 1H), 7.80-7.92 (m, 2H), 7.51-7.60 (m, 2H), 7.25 (t, *J*=7.72 Hz, 1H), 4.86 (q, *J*=7.03 Hz, 2H), 4.53 (br t, *J*=5.09 Hz, 2H), 3.68 (t, *J*=5.18 Hz, 2H), 3.11 (s, 3H), 2.89 (s, 3H), 1.42 (t, *J*=7.06 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 462.1584, found: 462.1583.

2-(9-Ethyl-8-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (39). **(**a) Methyl 2'-amino-6-fluoro-3'-methyl[biphenyl]-3-carboxylate (**127**). In analogy to intermediate **104**, 3.4 g (99%) of intermediate **127** were obtained from 2.2 g (14.57 mmol) of (2-amino-3-methylphenyl)boronic acid and 3.71 g (13.25 mmol) of methyl 4-fluoro-3-iodobenzoate. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.00 (ddd, *J*=2.45, 4.99, 8.57 Hz, 1H), 7.89 (dd,

J=2.26, 7.35 Hz, 1H), 7.43 (t, *J*=9.06 Hz, 1H), 6.97-7.07 (m, 1H), 6.76-6.88 (m, 1H), 6.54-6.63 (m, 1H), 4.46 (s, 2H), 3.81-3.91 (m, 3H), 2.11-2.14 (m, 3H).

(b) 8-Methyl-9*H*-carbazole-3-carboxylic acid (**128**). In analogy to intermediate **105**, 1.3 g (5.01 mmol) of intermediate **127** were treated with 702 mg of NaH (60%) to give 200 mg (18%) of intermediate **128**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.47 (br s, 1H), 11.57 (s, 1H), 8.71-8.75 (m, 1H), 7.97-8.08 (m, 2H), 7.54 (d, *J*=8.67 Hz, 1H), 7.19-7.29 (m, 1H), 7.04-7.19 (m, 1H), 2.52-2.59 (m, 3H).

(c) Ethyl 9-ethyl-8-methyl-9*H*-carbazole-3-carboxylate (**129**). In analogy to intermediate **106**, 200 mg (0.89 mmol) of intermediate **128** were treated with 0.35 mL (4.44 mmol) of ethyl iodide and 579 mg (1.78 mmol) of cesium carbonate to give 140 mg (56%) of intermediate **129**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.76 (d, *J*=1.32 Hz, 1H), 8.00-8.16 (m, 2H), 7.69 (d, *J*=8.67 Hz, 1H), 7.21-7.30 (m, 1H), 7.08-7.21 (m, 1H), 4.65 (q, *J*=7.10 Hz, 2H), 4.36 (q, *J*=7.16 Hz, 2H), 2.81 (s, 3H), 1.30-1.41 (m, 6H).

(d) 9-Ethyl-8-methyl-9*H*-carbazole-3-carboxylic acid (**130**). 550 mg of ethyl ester **129** were saponified in analogy to intermediate **107** using 9.8 mL of 2N NaOH to give 460 mg (93%) of the title compound **130**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.63 (br s, 1H), 8.75 (d, *J*=1.51 Hz, 1H), 7.96-8.13 (m, 2H), 7.67 (d, *J*=8.78 Hz, 1H), 7.12-7.28 (m, 2H), 4.65 (q, *J*=7.11 Hz, 2H), 2.81 (s, 3H), 1.35 (t, *J*=7.03 Hz, 3H).

(e) Methyl 2-(9-ethyl-8-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylate (**131**). In analogy to intermediate **120**, 75 mg (0.32 mmol) of intermediate **54** and 80 mg (0.32 mmol) of intermediate **130** gave 153 mg of crude material of

the title compound **131** which was used in the final saponification step without further purification.

(f) 2-(9-Ethyl-8-methyl-9*H*-carbazol-3-yl)-1-(2-methoxyethyl)-4-methyl-1*H*-benzimidazole-5-carboxylic acid (**39**). In analogy to compound **2**, 30 mg (0.07 mmol, 22% over 2 steps) of the title compound **39** were obtained by saponification of 153 mg of crude methyl ester **131** using 2N NaOH in methanol. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (br s, 1H), 8.61 (d, *J*=1.26 Hz, 1H), 8.11 (d, *J*=7.58 Hz, 1H), 7.76-7.93 (m, 3H), 7.53-7.59 (m, 1H), 7.26 (d, *J*=7.33 Hz, 1H), 7.14 (t, *J*=7.58 Hz, 1H), 4.69 (q, *J*=6.91 Hz, 2H), 4.53 (t, *J*=5.18 Hz, 2H), 3.63-3.78 (m, 2H), 3.12 (s, 3H), 2.89 (s, 3H), 2.83 (s, 3H), 1.38 (t, *J*=7.07 Hz, 3H); HRMS (ESI, $[M+H]^+$): calc.: 442.2131, found: 442.2130.

Ethyl 3-amino-4-[(2-methoxyethyl)amino]benzoate (75). Title compound **75** was prepared from ethyl 4-[(2-methoxyethyl)amino]-3-nitrobenzoate **(73)** by reduction with hydrogen on palladium analogously to 3-amino-4-[(2-methoxyethyl)amino]benzoic acid **(76)** from nitro compound **73**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (dd, *J*=1.64, 7.96 Hz, 1H), 6.71 (d, *J*=1.77 Hz, 1H), 6.17 (d, *J*=8.08 Hz, 1H), 6.02 (br s, 1H), 4.14-4.22 (m, 2H), 3.39-3.48 (m, 2H), 3.26-3.32 (m, 5H), 1.25 (t, *J*=7.07 Hz, 3H), NH2 not specified.

3-Amino-4-[(2-methoxyethyl)amino]benzoic acid (76). (a) Ethyl 4-[(2-methoxyethyl)amino]-3-nitrobenzoate (**73**). 40.0 g (0.17 mol) of ethyl 4-chloro-3-nitrobenzoate were added to 200 mL of DMSO, 20.9 g (0.28 mol) of 2-methoxyethanamine were added, and the mixture was heated for 6 h at 60 °C and then cooled to RT overnight. The reaction mixture was poured onto 200 mL of saturated sodium hydrogencarbonate solution, and the resulting precipitate was filtered off and washed with 100 mL of water. The precipitate was dried. 45.5 g (78%) of the title compound **73**

were thus obtained. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (d, *J*=2.07 Hz, 1H), 8.53 (br s, 1H), 7.96 (dd, *J*=1.60, 9.14 Hz, 1H), 7.17 (d, *J*=9.04 Hz, 1H), 4.29 (q, *J*=7.03 Hz, 2H), 3.55-3.64 (m, 4H), 3.29-3.33 (m, 3H), 1.31 (t, *J*=7.06 Hz, 3H).

(b) 4-[(2-Methoxyethyl)amino]-3-nitrobenzoic acid (**74**). 26.0 g (0.097 mol) of ethyl 4-[(2-methoxyethyl)amino]-3-nitrobenzoate **(73)** were added to 100 mL of ethanol, treated with 55 mL of 2 M NaOH solution and heated to reflux for 1 h. After cooling, 75 mL of 2 M HCl were added and the mixture was extracted five times with dichloromethane. The combined organic phases were dried over sodium sulfate, filtered and concentrated. 21.8 g (93%) of the title compound **74** were thus obtained. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.91 (br s, 1H), 8.61 (d, *J*=2.07 Hz, 1H), 8.50 (br s, 1H), 7.90-8.04 (m, 1H), 7.17 (d, *J*=9.04 Hz, 1H), 3.54-3.64 (m, 4H), 3.31 (s, 3H).

(c) 3-Amino-4-[(2-methoxyethyl)amino]benzoic acid (**76**). 21.8 g (0.09 mol) of 4-[(2-methoxyethyl)amino]-3-nitrobenzoic acid **(74)** were dissolved in 500 mL of ethanol, treated with 2.5 g of palladium/carbon (10%) and stirred at RT for 4 h with introduction of hydrogen. Then 2.5 g of palladium/carbon (10%) were added once again and hydrogen was introduced for a further 2 h. The catalyst was filtered off and the solution was concentrated. 19.0 g (82%) of the title compound **67** were thus obtained. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (br s, 1H), 7.12-7.20 (m, 1H), 6.65-6.74 (m, 1H), 6.08-6.20 (m, 1H), 5.42-6.06 (m, 1H), 5.00-6.06 (m, 1H), 3.42-3.47 (m, 2H), 3.24-3.29 (m, 5H), NH not stated.

Pharmcology. Compound logistics. Ready to use test plates were prepared in advance for all assays used in screening, hit confirmation and compound characterization of the human prostaglandin EP4-, EP2- and DP receptors. Fifty nanoliter of a 100-fold concentrated solution of

the test compound (in 100 % DMSO) were transferred into a white, small volume microtiter plate (Greiner Bio-One, Germany) using either a Hummingbird liquid handler (Digilab, MA, USA) or an Echo acoustic system (Labcyte, CA, USA). Negative and positive control wells, typically 16 wells in a 384 well plate or 64 wells in a 1536 well plate, received 50 nanoliter of 100 % DMSO only. A 10 µM compound concentration was used for screening purposes followed by single concentration confirmation testing in duplicates at 25 and 5 µM. For the establishment of dose response curves compounds were typically tested in duplicates at up to 11 concentrations (for example 20 µM, 5.7 µM, 1.6 µM, 0.47 µM, 0.13 µM, 38 nM, 11 nM, 3.1 nM, 0.89 nM, 0.25 nM and 0.073 nM).

Compounds used in species specificity testing were always prepared fresh starting from a 10 mM solution (in 100% DMSO) and initially diluted 1:10 in 100% DMSO to obtain a 1 mM stock solution. Next, six microliter of this stock solution were transferred into 300 microliter PBS to generate a 20 µM test solution (in 2% DMSO) used as starting point for serial dilutions prepared shortly after. A Tecan Freedom Evo instrument (Tecan, Germany) was used to transfer 60 microliter thereof into a white, 96-well dilution plate (ThermoFischer Scientific, Germany, Nunc, PP, #249944) containing 200 microliter of PBS / 2 % DMSO already in order to prepare a first 1:3.3 dilution followed by additional nine alternating 1:3 / 1:3.3 dilution steps (i.e. 60 microliter / 67 microliter respectively being transferred to the next well containing 200 microliter PBS/2 % DMSO). A total of 10 serial dilutions were prepared containing a two-fold concentration of test compound (i.e. 6.0 µM, 2.0 µM, 600nM, 200 nM, 60 nM, 20 nM, 6.0 nM, 2.0 nM, 0.6 nM, and 0.2 nM). Finally, 5 microliter thereof were transferred to a black, small volume 384-well compound test plate (Greiner BioOne, #784076) to be tested in quadruplicates

(CyBi Well Vario, Analytik Jena, Germany). Negative and positive control wells received 5 microliter of PBS / 2% DMSO only.

Frozen cell assays. All cell lines used were routinely monitored for the presence of mycoplasma and shown to be free of any contamination. Vials containing the cell line expressing the appropriate receptor were removed from liquid nitrogen storage and rapidly thawed by placing them into a 37°C water bath. Immediately after thawing the cells were transferred by gently decanting into a 50 mL Falcon tube containing preheated medium (see actual assay protocols for details). Any liquid remaining in the vials was softly rinsed with preheated medium and transferred into the falcon tube as well. Next, the cells were harvested by centrifugation for 5 minutes at 18.0 x g and the supernatant removed by gentle decanting prior to slowly adding preheated medium again and resuspending the pellet by mild swirling. A small aliquot was removed for cell counting and the cells were diluted to the final cell number (see assay protocols). Finally, an aliquot of the cAMP-d2 stock solution (prepared according the manufacturers protocol) was added to achieve a 1:40 dilution step. The cells were used for seeding into assay plates right away as no additional preincubation step was necessary to achieve full functionality.

General assay principle. Agonist induced activation of the human, mouse, rat and cynomolgous EP4 receptor (h, m, r, and cEP4-R) as well as the human EP2 (hEP2-R) and the human DP receptor (hDP-R) results in the production of cAMP, which – upon cell lysis – competes with a fluorescent cAMP-tracer (cAMP-d2) for the binding to an Eu-cryptate labelled anti-CAMP antibody (CisBio International; HTRF cAMP Assay, see detailed assay descriptions below). Maximum signal resulting from fluorescence resonance energy transfer (FRET) between the detection reagents is obtained in the absence of cellular cAMP. Any decrease in FRET signal is

indicative of hEP4-R activation whereas antagonist activity results in signal increase once again. FRET signal quantification is achieved with the help of an appropriate plate reader (PheraStar; RubyStar; ViewLux). Following excitation at 337 nm any reduction of FRET induced emissions at 665 nm is indicative of agonist induced cAMP production. In addition, a second FRET signal at 620 nm, originating from the Eu-labelled Anti cAMP antibody, is used for well internal referencing (Well-Ratio; defined as 665nm/620nm*10000).

General assay procedures. All steps of the actual assays were performed at room temperature and all solutions were added with the help of a Multidrop dispenser (Thermo Labsystems).

Data Analysis. Screen results were analyzed and both, EC50 and IC50 values were either calculated by 4-Parameter fitting using a commercial software package (Genedata Screener, Switzerland) or proprietary software developed in-house.

Cellular hEP4-R assay. Typically, the reaction volume was 6 microliter in both 1536 and 384 well plates. Four microliters of the cell suspension containing 625 cells/microliter (in Ham´s F12 supplemented with 5 % charcoal treated serum) were added to all wells of the ready to use test plate. Following a 20 minute preincubation two microliters of a $3XEC_{80}$ PGE2 agonist solution (1.20E-9M; prepared fresh in Ham´s-F12, 5 % charcoal treated serum; 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received Ham´s-F12, 5 % charcoal treated serum, 0.75 mM IBMX only. Following that, the plate was incubated for another 60 minutes in the presence of a 1XEC80 concentration of agonist (4.00E-10M). The reaction was stopped by the addition of 2 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers protocol (cAMP-Gs dynamic kit). Another

20 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader in order to quantify the results.

Cellular hEP2-R selectivity testing. The reaction volume was 6 microliter in 384 well plates. Four microliters of the cell suspension containing 625 cells/microliter (in DMEM high Glucose supplemented with 5 % charcoal treated serum) were added to all wells of the ready to use test plate. Following a 20 minute preincubation two microliters of a $3XEC_{80}$ PGE2 agonist solution (1.50E-9M; prepared fresh in DMEM high Glucose supplemented with 5 % charcoal treated serum; 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received DMEM high Glucose, 5 % charcoal treated serum, 0.75 mM IBMX only. Following that, the plate was incubated for another 60 minutes in the presence of a 1XEC80 concentration of agonist (5.00E-10M). The reaction was stopped by the addition of 2 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers protocol (cAMP-Gs HiRange). Another 20 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader in order to quantify the results.

Cellular hDP-R selectivity testing. Again, the reaction volume was 6 microliter in 384 well plates. Four microliters of the cell suspension containing 625 cells/microliter (in DMEM high Glucose supplemented with 5 % charcoal treated serum) were added to all wells of the ready to use test plate. Following a 20 minute preincubation at room temperature two microliters of a $3XEC_{80}$ PGD_2 agonist solution (1.50E-9M; prepared fresh in DMEM high Glucose supplemented with 5 % charcoal treated serum; 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received DMEM high Glucose, 5 % charcoal treated serum, 0.75 mM IBMX

only. Following that, the plate was incubated for another 60 minutes in the presence of a 1XEC80 concentration of agonist (5.00E-10M). The reaction was stopped by the addition of 2 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers protocol (cAMP-Gs HiRange kit). Another 20 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader in order to quantify the results.

Cellular mouse EP4 receptor species specificity assay. The reaction volume was 20 microliter in 384 well plates. CHO-cells stably expressing the mouse EP4-R (obtained from Prof. Y. Sugimoto) were harvested from continuous culture (i.e. MEM-Alpha medium without nucleosides, PAA Laboratories, #E15-832; 10 % FCS, Biochrom, S 0115; 100 Units Penicillin/0.1 mg/mL Streptomycin, Sigma-Aldrich, P4333) by Accutase treatment (Sigma-Aldrich, A6964) and used to prepare a cell suspension of 2 000 cells/microliter in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX supplemented with a 1:40 dilution of cAMP-d2 solution prepared according to the manufacturers protocol. Five microliters of the cell suspension (i.e. 10000 cells/well) were added to all wells of the compound test plate using a MultiDrop™ Combi instrument (ThermoFischer Scientific, Germany). Following a 30 minute preincubation at room temperature 5 microliters of a $3XEC_{80}$ PGE2 agonist solution (3.00E-8 M; prepared fresh in in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX only. Following that, the plate was incubated for another 30 minutes in the presence of a 1XEC80 concentration of agonist (1.00E-8M). The reaction was stopped by the addition of 5 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers

protocol (cAMP-Gs HiRange). Another 90 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader (PheraStar Plus, BMG Labtech, Germany) in order to quantify the results.

Cellular rat EP4 receptor species specificity assay. HEK293 human embryonic kidney cells (ATCC® CRL-1573™) transiently expressing the rat EP4-R were prepared using a cationic liposome formulation. Briefly, plasmid DNA-lipid complexes were formed by dropwise addition of 2.5 mL of Opti-MEM containing 20 microliter of an expression vector for rEP4-R (pGT-414 ratEP4, 10 nanogram/microliter) to a second vial containing 2.5 mL of Opti-MEM and 60 microliter of Lipofectamine™ (ThermoFischer Scientific, Cat. No. 11686-019) followed by an incubation for 45 minutes. HEK293 grown to 80% confluency in 162 cm^2 flasks were washed once with Opti-MEM prior to the addition of 15 mL OptiMEM medium followed by dropwise addition of 5 mL OptiMEM containing the DNA-lipid complexes. The cells supernatant was removed following a 5 hour incubation at 37°C, 7 % CO_2 and replaced by 20 mL DMEM medium containing 5 % charcoal treated serum for overnight incubation at 37°C, 7 % CO_2. The cells were harvested by Accutase treatment (Sigma-Aldrich, A6964) the next day and used to prepare a cell suspension of 2 000 cells/microliter in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX supplemented with a 1:40 dilution of cAMP-d2 solution prepared according to the manufacturers protocol. Five microliters of this cell suspension (i.e. 10000 cells/well) were added to all wells of the compound test plate using a MultiDrop™ Combi instrument (ThermoFischer Scientific, Germany). Following a 30 minute preincubation 5 microliters of a $3XEC_{80}$ PGE2 agonist solution (3.00E-9 M; prepared fresh in in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received PBS, 0.1% BSA, 0.2%

Glucose, 2.25 mM IBMX only. Following that, the plate was incubated for another 30 minutes in the presence of a 1XEC80 concentration of agonist (1.00E-9M). The reaction was stopped by the addition 5 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers protocol. Another 90 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader (PheraStar Plus, BMG Labtech, Germany) in order to quantify the results.

Cellular cynomolgous monkey EP4-R species specificity assay. HEK293 human embryonic kidney cells (ATCC® CRL-1573™) transiently expressing the cynomolgous monkey EP4 receptor (cEP4-R) were prepared using a cationic liposome formulation. Plasmid DNA-lipid complexes were formed by dropwise addition of 2.5 mL of Opti-MEM containing 20 microliter of an expression vector for rEP4-R (pGT-414 ratEP4, 10 nanogram/microliter) to a second vial containing 2.5 mL of Opti-MEM and 60 microliter of Lipofectamine™ (ThermoFischer Scientific, Cat. No. 11686-019) followed by an incubation for 45 minutes. HEK293 grown to 80% confluency in 162 cm^2 flasks were washed once with Opti-MEM prior to the addition of 15 mL OptiMEM medium followed by dropwise addition of 5 mL OptiMEM containing the DNA-lipid complexes. The cells supernatant was removed following a 5 hour incubation at 37°C, 7 % CO_2 and replaced by 20 mL DMEM medium containing 5 % charcoal treated serum for overnight incubation at 37°C, 7 % CO_2. The cells were harvested by Accutase treatment (Sigma-Aldrich, A6964) the next day and used to prepare a cell suspension of 2 000 cells/microliter in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX supplemented with a 1:40 dilution of cAMP-d2 solution prepared according to the manufacturers protocol. Five microliters of this cell suspension (i.e. 10000 cells/well) were added to all wells of the compound test plate using a MultiDrop™ Combi instrument (ThermoFischer Scientific, Germany). Following a 30 minute

preincubation 5 microliters of a $3XEC_{80}$ PGE2 agonist solution (3.00E-9 M; prepared fresh in in PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX) was added to the test compound and positive control wells (low controls [C(0)]). The negative control wells (high controls, [C(i)]) received PBS, 0.1% BSA, 0.2% Glucose, 2.25 mM IBMX only. Following that, the plate was incubated for another 30 minutes in the presence of a 1XEC80 concentration of agonist (1.00E-9M). The reaction was stopped by the addition 5 microliter of lysis buffer containing a 1:20 dilution of the Eu-cryptate labelled anti-CAMP antibody stock prepared according the manufacturers protocol. Another 90 minutes later the cell lysate containing plate is transferred to TR-FRET compatible reader (PheraStar Plus, BMG Labtech, Germany) in order to quantify the results.

Metabolite profiling in human and animal hepatocytes. Preparations of hepatocytes in Williams' E Medium (suspension culture freshly prepared according to standard procedure) from rat, monkey, dog and man were incubated at 37 °C with 10 μM compound **13**, **28**, **32** for 4h. Compounds **13**, **28**, **32** were added from a 0.1 mM stock solution dissolved in acetonitrile to the incubation mixture.

The incubations were terminated by the addition of acetonitrile (approx. 30% (v/v)) at time points 0, 1, 2 and 4h. The samples were stored at 20 °C until analysis. Prior to analysis the samples were thawed and centrifuged at 450 U/min for 5 min. The supernatants were removed and an aliquot of 10 microliter was used to control the recovery of radioactivity by liquid scintillation counting. Further aliquots of the supernatants were analyzed by HPLC with UV and mass spectrometric detection.

Investigation of *in vitro* metabolic stability in rat hepatocytes. Hepatocytes from Han Wistar rats were isolated via a 2-step perfusion method. After perfusion, the liver was carefully removed from the rat: the liver capsule was opened and the hepatocytes were gently shaken out into a

Petri dish with ice-cold Williams' medium E (WME). The resulting cell suspension was filtered through sterile gaze in 50 mL falcon tubes and centrifuged at $50 \times g$ for 3 min at room temperature. The cell pellet was resuspended in 30 mL of WME and centrifuged through a Percoll® gradient for 2 times at $100 \times g$. The hepatocytes were washed again with WME and resuspended in medium containing 5% FCS. Cell viability was determined by trypan blue exclusion.

For the metabolic stability assay liver cells were distributed in WME containing 5% FCS to glass vials at a density of 1.0×10^6 vital cells/mL. The test compound was added to a final concentration of 1 µM. During incubation, the hepatocyte suspensions were continuously shaken at 580 rpm and aliquots were taken at 2, 8, 16, 30, 45 and 90 min, to which equal volumes of cold methanol were immediately added. Samples were frozen at -20° C over night, subsequently centrifuged for 15 minutes at 3000 rpm and the supernatant was analyzed with an Agilent 1200 HPLC-system with LCMS/MS detection.

The half-life of a test compound was determined from the concentration-time plot. From the half-life the intrinsic clearances were calculated using the `well stirred´ liver model together with the additional parameters liver blood flow, amount of liver cells *in vivo* and *in vitro.* The hepatic *in vivo* blood clearance (CL) and the maximal oral bioavailability (F_{max}) was calculated. The following parameter values were used: Liver blood flow – 4.2 L/h/kg rat; specific liver weight – 32 g/kg rat body weight; liver cells *in vivo*- 1.1 x 10^8 cells/g liver, liver cells *in vitro* – 1.0 x 10^6/mL.

***In vivo* pharmacokinetics in rats.** All animal studies have been performed in accordance with institutional guidelines as defined by Institutional Animal Care and the German protection of animals Act. For *in vivo* pharmacokinetic experiments test compounds were administered to

male Wistar rats intravenously at doses of 0.3 to 1 mg/kg and intragastral at doses of 0.5 to 10 mg/kg formulated as solutions using solubilizers such as PEG400 in well-tolerated amounts. In the rat also cassette administrations of up to 3 compounds given together in low doses were performed. Blood samples were collected e.g. at 2 min, 8 min, 15 min, 30 min, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h after dosing from the vena jugularis into Lithium-Heparin tubes (Monovetten®, Sarstedt) and centrifuged for 15 min at 3000 rpm. An aliquot of 100 microliter from the supernatant (plasma) was taken and precipitated by addition of 400 microliter cold acetonitril and frozen at -20°C over night. Samples were subsequently thawed and centrifuged at 3000 rpm, 4°C for 20 minutes. Aliquots of the supernatants were taken for analytical testing using an Agilent HPLC-system with LCMS/MS detection. PK parameters were calculated by non-compartmental analysis using a PK calculation software (e.g. Phoenix WinNonlin®, Certara USA, Inc.).

Caco-2 Permeation Assay. Caco-2 cells (purchased from DSMZ Braunschweig, Germany) were seeded at a density of 4.5 x 10^4 cell per well on 24 well insert plates, 0.4 µm pore size, and grown for 15 days in DMEM medium supplemented with 10% fetal bovine serum, 1% GlutaMAX (100x, GIBCO), 100 U/mL penicillin, 100µg/mL streptomycin (GIBCO) and 1% non-essential amino acids (100 x). Cells were maintained at 37°C in a humified 5% CO_2 atmosphere. Medium was changed every 2-3 day. Before running the permeation assay, the culture medium was replaced by an FCS-free Hepes-carbonate transport puffer (pH 7.2). For assessment of monolayer integrity the transepithelial electrical resistance (TEER) was measured. Test compounds were predissolved in DMSO and added either to the apical or basolateral compartment in final concentration of 2 µM. Before and after 2h incubation at 37°C samples were taken from both compartments. Analysis of compound content was done after precipitation

with methanol by LC/MS/MS analysis. Permeability (Papp) was calculated in the apical to basolateral (A → B) and basolateral to apical (B → A) directions. The apparent permeability was calculated using following equation: $P_{app} = (V_r/P_o)(1/S)(P_2/t)$ Where V_r is the volume of medium in the receiver chamber, P_o is the measured peak area of the test drug in the donor chamber at t=o, S the surface area of the monolayer, P_2 is the measured peak area of the test drug in the acceptor chamber after 2h of incubation, and t is the incubation time. The efflux ratio basolateral (B) to apical (A) was calculated by dividing the P_{app} B-A by the P_{app} A-B. In addition the compound recovery was calculated. As assay control reference compounds were analyzed in parallel.

Estimation of Plasma Protein Binding by Equilibrium Dialysis. Binding of test compounds to plasma proteins is measured by equilibrium dialysis in a 96-well format using ht-dialysis equipment made of Teflon and a semipermeable membrane (regenerated cellulose, MWCO 12-14K). The membrane separates the plasma and buffer side (50 mM phosphate buffer) filled with 150 microliter each. The test compound is added in a concentration of 3 µM to the plasma side and binds to plasma proteins. The unbound fraction of the test compound passes the membrane and distributes on both sides until equilibrium is reached, which is usually the case after 6-8h at 37°C. Compound concentration of plasma and buffer side is measured by LC-MSMS analytics. For this both sides are diluted with buffer and plasma to achieve the same matrix (10% plasma) and subsequently are precipitated with methanol. From the quotient of buffer and plasma concentration the free (unbound) fraction (fu) is calculated. Stability and recovery controls are included. Additionally, the test compound is dialyzed in buffer against buffer in order to estimate non-specific binding to equipment and/or membrane and to investigate in the establishment of the equilibrium. Due to the osmotic pressure of the plasma proteins a dilution of the plasma takes

place during the incubation (volume shift). The potential imprecision is addressed by inclusion of an empirical factor in the calculation of the fu. Establishment of equilibrium and stability in plasma should be at least 80% and the recovery in plasma should at least be 30%. A free fraction of <1% is designated as high, between 1 and 10% as moderate and of >10% as low plasma protein binding.

***In Vivo* characterization of dmPGE$_2$ induced pain behavior in rats.** *In vivo* experiments were performed by American Preclinical Services (APS), a contract research organization located in Minneapolis, USA providing an animal facility accredited by the association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) which follows the guidelines of the International Association for the Study of Pain for use of Animals in Research. The facilities were reviewed and approved by the American Preclinical Services Institutional Animal Care and Use Committee (IACUC) prior to study initiation. The actual animal model involves a single injection of dmPGE2 into the hind paw followed by measuring any pain related behavior by quantifying the withdrawal reaction of the paw in response to increasing mechanical stimulation. Animals: Male adult Sprague-Dawley rats (Harlan, Indianapolis, USA) weighing at the procedure 220-265 g were housed in groups of 3/cage. Animals were acclimated to the facility conditions (12 h :12 h light:dark cycle, with free access to food and water, approximately 70°F and 50% relative humidity) for a minimum of 3 days prior to the execution of any study-related activities. Drugs: Desired amount of 0.5% carboxymethylcellulose (CMC) (Sigma-Aldrich, St Louis, USA) solution was added to pre-weighed amount of **BAY 1316957** to produce a concentration of 1 mg/mL stock solution. The stock solution was further diluted with 0.5% CMC to desired concentration (0.04 and 0.2 mg/mL). Treatment. **BAY 1316957** was applied p.o. 60 minutes before application of dmPGE2 in a volume of 5 mL kg^{-1} and in three doses (0.2 / 1.0 /

5.0 mg kg^{-1}). Inflammatory pain was induced by a single injection of 10 µg 16,16-dimethyl prostaglandin E2 (dmPGE2; Cayman Chemicals, Ann Arbor, USA) in PBS (pH between 7.0 to 7.4) into the plantar surface of the left hind paw. Measurement: The behavioral response to mechanical stimulation of the paws was measured using an electronic von Frey apparatus (eVF, IITC Life Sciences, Woodland Hills, USA). In detail: Animals were placed in individual acrylic chambers on a metal mesh surface and allowed to acclimate to their surroundings for a minimum of 15 minutes before testing. The stimulus was presented perpendicular to the plantar surface and pressure was applied gradually. Paw withdrawal threshold values were recorded when a positive response was noted (paw sharply withdrawn) or the paw is lifted off the mesh surface. Three eVF thresholds were measured for each hind paw per time point. The mean of the 3 values was taken as the paw withdrawal threshold for that time point. The mean and standard deviation (SD) were determined for each paw for each treatment group at each time point. After the naïve baseline values for paw withdrawal thresholds were obtained on day -1, animals were assigned to different treatment and control groups and received test article or vehicle administration (day 0, T=0). Approximately 60 minutes after the treatment (day 0, T=60 min), inflammatory pain was induced by a single injection of 10 µg dmPGE2 into the plantar surface of the left hind paw. Paw withdrawal thresholds were then measured 30 minutes after dmPGE2 injection (day 0, T=90 min). All animals were euthanized on day 0 following the behavioral assessment and plasma was collected from animals treated with test articles. Statistical analysis: Data are plotted as mean ±SD. The Grubbs test was used in order to distinguish potential outliers. For analysis of all experiments 1-way ANOVA followed by Dunnett´s post-hoc test versus control were performed. GraphPad Software Inc., La Jolla, USA, was used for all analysis and statistical calculations.

Off-target assay panels. Compounds were tested in customized binding studies based on Ricerca's (now Eurofins) LeadProfiler Screen comprising a panel of 77 GPCRs, ion channels, kinases and transporters screened at 10 μM. With the exception of the DP2-R all other members of the human prostanoid receptor family were checked for agonistic and antagonistic activity either in-house or based on Millipore´s (now Eurofins) functional GPCRProfiler service. A total of 25 pharmacologically relevant GPCR´s known to represent frequent off-targets in GPCR screening were checked at 10 μM again ("BAYER-GPCR Panel").

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

With the exception of D.W. (Evotec (UK) Ltd) and D.H. all authors are employees of Bayer AG and members of the respective research labs involved in the generation of these results.

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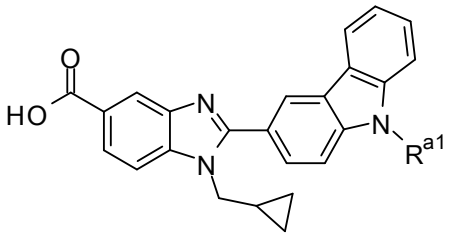
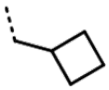
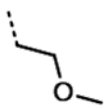
The authors thank Marco Sommer and Kai Steger (Lead Discovery Berlin, Screening Cell Biology) as well as Martina Backhus, Michaela Niefer and Ulrike Konrad (Gynecological Therapies, Berlin) and Elke Ducke, Carsten Schreier, Agnes Spranger and Sabine Weber (Medicinal Chemistry, Berlin) for their excellent technical assistance throughout the whole project. In addition, Gisbert Depke is acknowledged for excellent analytical support.

Tables

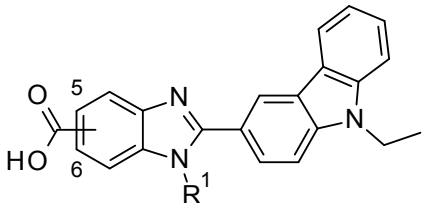
Table 1. hEP4-R Antagonism: Effect of variations of substituents at the carbazole nitrogen

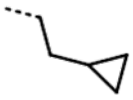
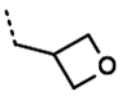
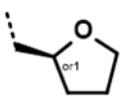


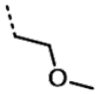
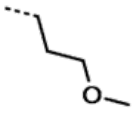
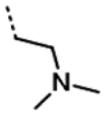
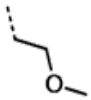
compd	R^{a1}	IC_{50} hEP4-R [nM][a]	IC_{50} hEP4-R (Stdev [nM])
2	Et	12.8	± 8.2
3	Me	34.2	± 25
4	*n*-Pr	63	± 3.2
5		33.8	± 13.2
6		257.1	± 97.5
7		18	± 2.4

[a] Values represent averages of at least two independent experiments done in duplicates, unless noted otherwise

Table 2. hEP4-R Antagonism. Substitutions of the benzimidazole-nitrogen leading to the identification of compound 13 as lead structure

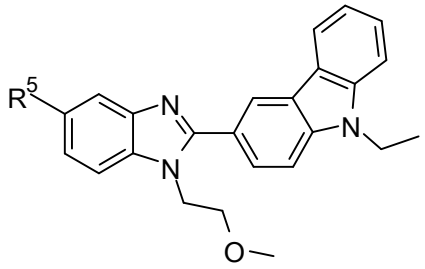


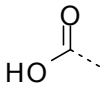
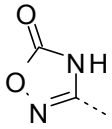
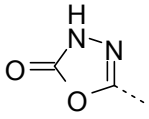
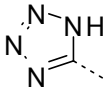
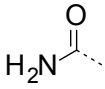
compd	Position carboxylate	R^1	IC_{50} hEP4-R [nM][a]	IC_{50} hEP4-R (Stdev [nM])
2	5		12.8	± 8.2
8	5		22.1	0
9	5	*i*-Pr	733.3	± 122
10	5	Me	165.5	± 112.9
11	5		51.5	± 9.2
12	5		7	± 2.1

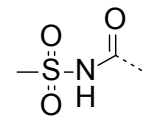
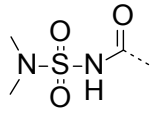
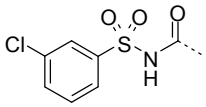
13	5		4.7	± 3.1
14	5		157.7[b]	-
15	5		82.5	± 6.8
16	5	Ph	3118[b]	-
17	6		1422	± 137.2

[a] Values represent averages of at least two independent experiments done in duplicates, unless noted otherwise. [b] = single result.

Table 3. Effects of bioisosteric replacements of the carboxylic acid moiety (R1) in compound 13 on hEP4-R antagonism and pharmacokinetic properties



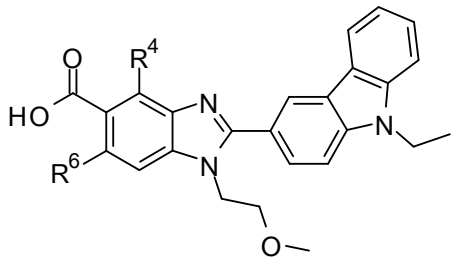
cmpd	R^5	IC_{50} hEP4-R [nM][a]	Stdev [nM]	PK Caco-2 P_{app} A-B [nm s^{-1}]	Efflux Ratio	PK Hep CL_{blood} [L h^{-1} kg^{-1}]	F_{max}[b] [%]
13		4.7	± 3.1	206	0.6	3.3	22
18		6.1	± 4.6	26.3	3.3	0.7	84
19		43.7	± 5	12.4	0.9	1.8	57
20		6.8	± 4.7	7.4	15	1.9	54
21		134.2	± 27.9	102.4	0.8	2.5	40

22		23.8	± 11	0	0	1.6	61
23		147.7	0	nd	nd	nd	nd
24		221.9	± 84	nd	nd	nd	nd
25		14.7	± 3.2	1.6	56	0.3	92

[a] Values represent averages of at least two independent experiments done in duplicates unless noted otherwise. **nd** = not determined.

[b] Calculated maximal oral bioavailability F_{max} = 1 – (blood clearance divided by species specific liver blood flow)

Table 4. Effects of *ortho*-substitution of 13 on hEP4-R potency and pharmacokinetic properties *in vitro*

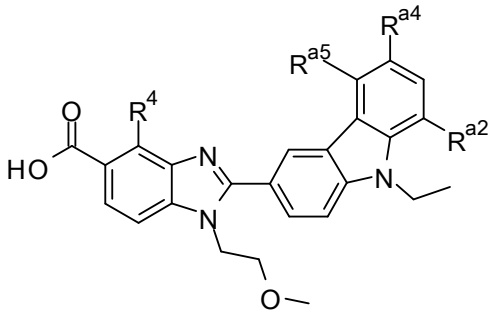


compd	R^4	R^6	IC_{50} hEP4-R [nM][a]	Stdev [nM]	PK Caco-2 Papp A-B [nm s^{-1}]	Efflux Ratio	PK Hep Cl CL_{blood} [L h^{-1} kg^{-1}]	F_{max}[b] [%]
13	H	H	4.7	± 3.1	206	0.6	3.3	22
26	F	H	4.3	± 2.3	10.4	18.6	2.6	38
27	Cl	H	15.9	± 3.1	27.6	6.6	1.2	72
28	Me	H	24.7	± 23.2	135.4	1.1	0.1	96
29	H	F	34.6	± 15.9	12.4	17.1	0.9	79.9
30	H	Me	611.7	± 209.5	98.1	1.5	3.5	16
31	H	Cl	751.1	± 29.9	nd	nd	nd	nd

[a] Values represent averages of at least two independent experiments done in duplicates unless noted otherwise. Nd = not determined

[b] Calculated maximal oral bioavailability F_{max} = 1 – (blood clearance divided by species specific liver blood flow)

Table 5. Carbazole SAR - identification of BAY 1316957



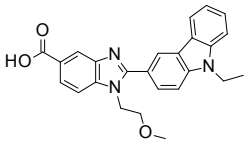
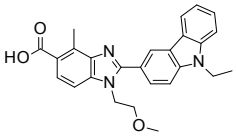
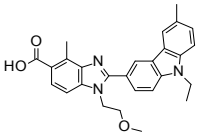
compd	R^4	R^{a4}	R^{a2}	R^{a5}	IC50 hEP4-R [nM][a]	Stdev [nM]	PK Caco-2 Papp A-B [nm s^{-1}]	Efflux Ratio	PK Hep Cl CL_{blood} [L h^{-1} kg^{-1}]	F_{max}[b] [%]
13	H	H	H	H	4.7	± 3.1	206	0.6	3.3	22
28	Me	H	H	H	24.7	± 23.2	135.4	1.1	0.1	96
32	Me	Me	H	H	15.3	± 10.7	205.9	0.6	0.4	90
33	Me	Cl	H	H	4.4	± 2.6	208.4	0.5	0.6	86
34	Me	CF_3	H	H	17.4	± 1.1	195.4	0.5	1.1	74
35	Me	OCF_3	H	H	365.6	± 40.7	nd	nd	nd	nd
36	Me	H	H	Cl	65.5	± 3.7	169.1	0.6	0.5	88

37	Me	H	H	Me	54.6	± 2.5	nd	nd	nd	nd
38	Me	H	Cl	H	5.2	± 1.7	206.5	0.4	0.6	86
39	Me	H	Me	H	4.2	± 0.7	178.5	0.7	1.4	67

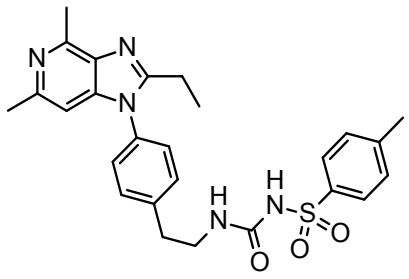
[a] Values represent averages of at least two independent experiments done in duplicates unless noted otherwise

[b] Calculated maximal oral bioavailability F_{max} = 1 – (blood clearance divided by species specific liver blood flow)

Table 6. **Pharmacokinetic parameters of the lead structure, the optimized lead and the final candidate (i.e. compounds: 13, 28 and 32)**

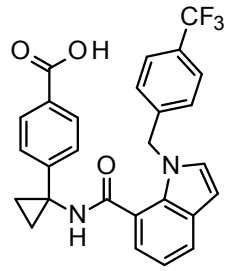
							
		13		**28**		**32**	
		i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
Dosing (Wistar rats)	[mg kg^{-1}]	0.37	0.38	1.0	1.0	1.0	0.5
$t_{1/2}$	[h]	2.6		4.9		4.9	
AUC_{norm}	[kg h L^{-1}]		2.6		20.0		24.0
Cl_{blood}	[L h^{-1} kg^{-1}]	0.32		0.06		0.06	
V_{ss}	[L kg^{-1}]	0.82		0.36		0.24	
F	[%]	48		92		90	
Intrinsic Cl_{blood} (rat hepatocytes)	[L h^{-1} kg^{-1}]	2.8		0.15		0.43	
Caco-2 permeation	Efflux ratio	1.6		1.1		0.6	
Solubility pH 6.5 (kinetic)	[mg L^{-1}]	191		150		143	

Figures

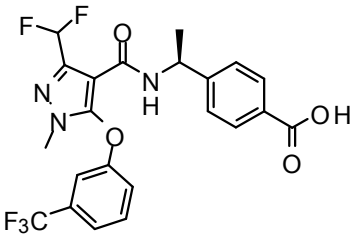


Raqualia07

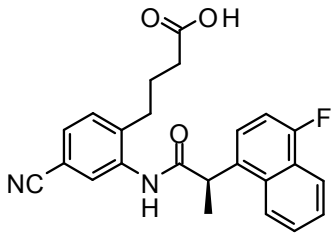
Grapiprant



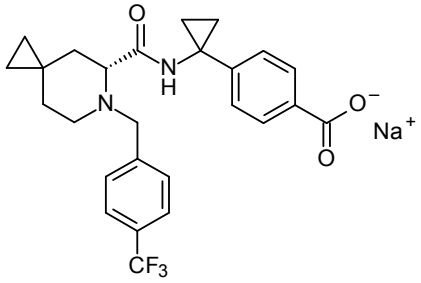
Merck-Frosst MF-766



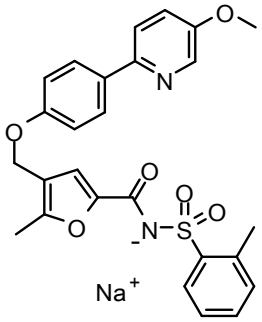
Eisai E-7046



ONO-AE3-208



CR-6086



BGC20-1531

Figure 1. Selected published structures of clinical hEP4-R antagonists

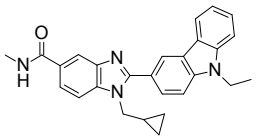
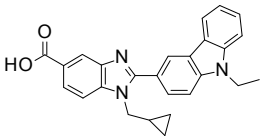
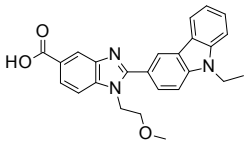
	Screening hit	Lead-like structure	Lead structure
			
cmpd	**1**	**2**	**13**
IC_{50} hEP4-R	286.1 nM	12.8 nM	4.7 nM
IC_{50} mEP4-R	nd	113 nM	81 nM
Lipophilicity (logD)	4.1	3.0	2.0
Solubility (S_w kin.)	< 0.1 mg L^{-1}	3.2 mg L^{-1}	137 mg L^{-1}

Figure 2. Key compounds identified in the hit to lead phase. Early replacement of the N-methyl carboxamide in hit compound **1** by a free carboxylic acid (compound **2)** improved hEP4-R IC_{50} by approximately 26-fold and subsequent derivatizations of the benzimidazole-nitrogen in **2** led to the identification of the methoxyethyl moiety, providing lead compound **13**.

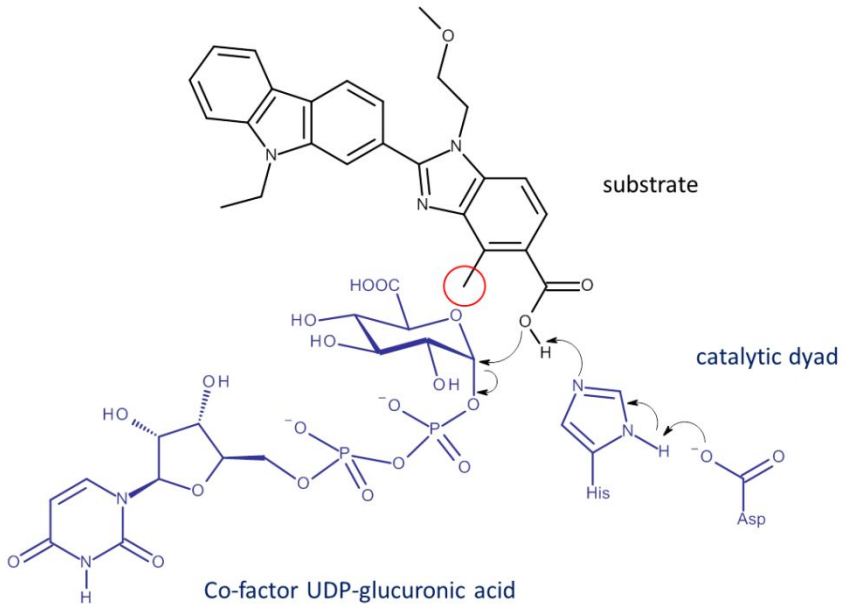


Figure 3. The proposed catalytic mechanism for O-glucuronidation (so-called "serine hydrolase like mechanism") by human UGTs adopted from Dong et al. (2012) involves a catalytic dyad of two key amino acids histidine and aspartic acid. Small substituents neighbouring the carboxylate moiety in the substrate (red circle, as in **28**) may "detoxify" the hEP4-R lead structure compound **13** by prohibiting Phase II metabolism either by clashing with the protein or the co-factor or by changing the orientation and local structure of the nucleophilic atom.

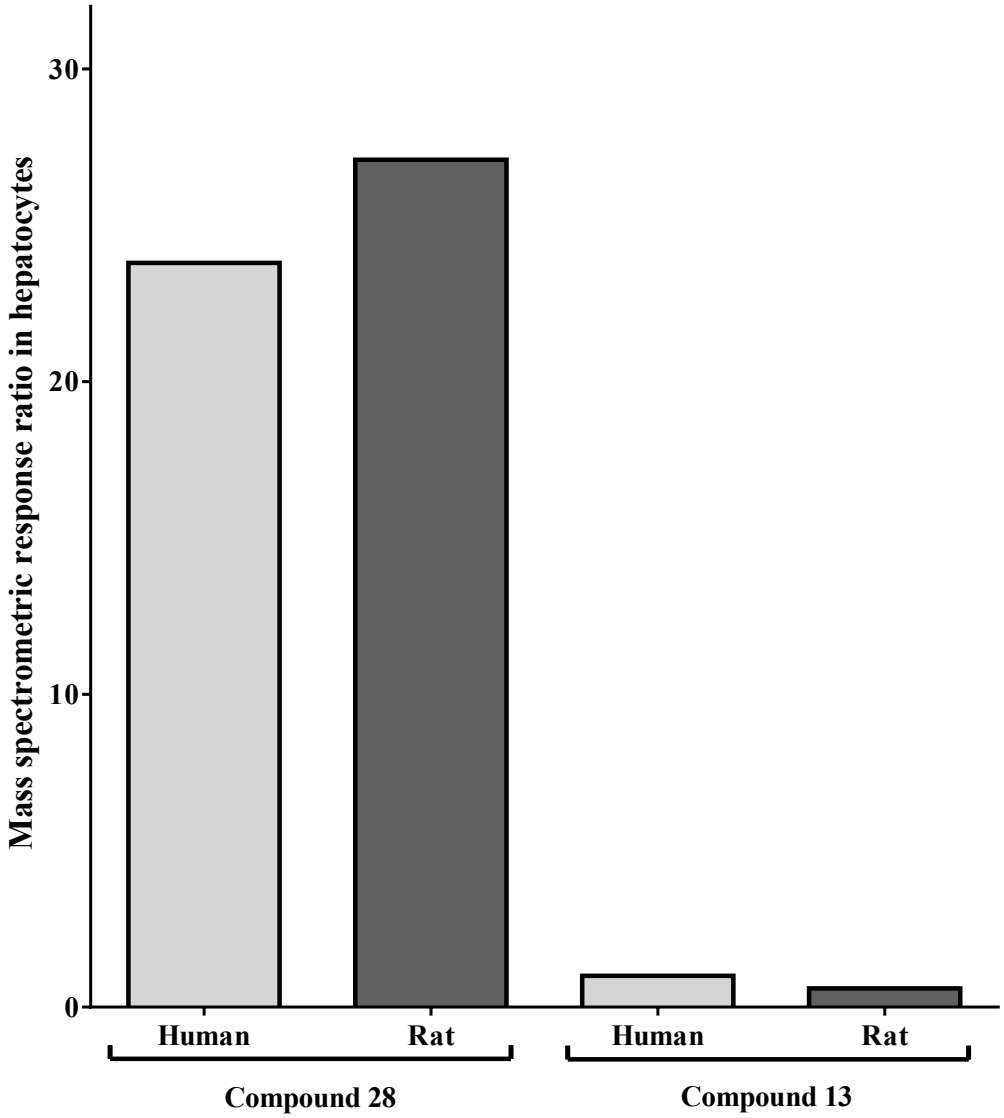


Figure 4. Ratio of mass spectrometric responses obtained for parent (lead compound **13)** and methylated compound **28** to their respective metabolites (corresponding acylglucuronides); data for human and rat hepatocytes shown, following 4 hours of incubation of 10 μM compound.

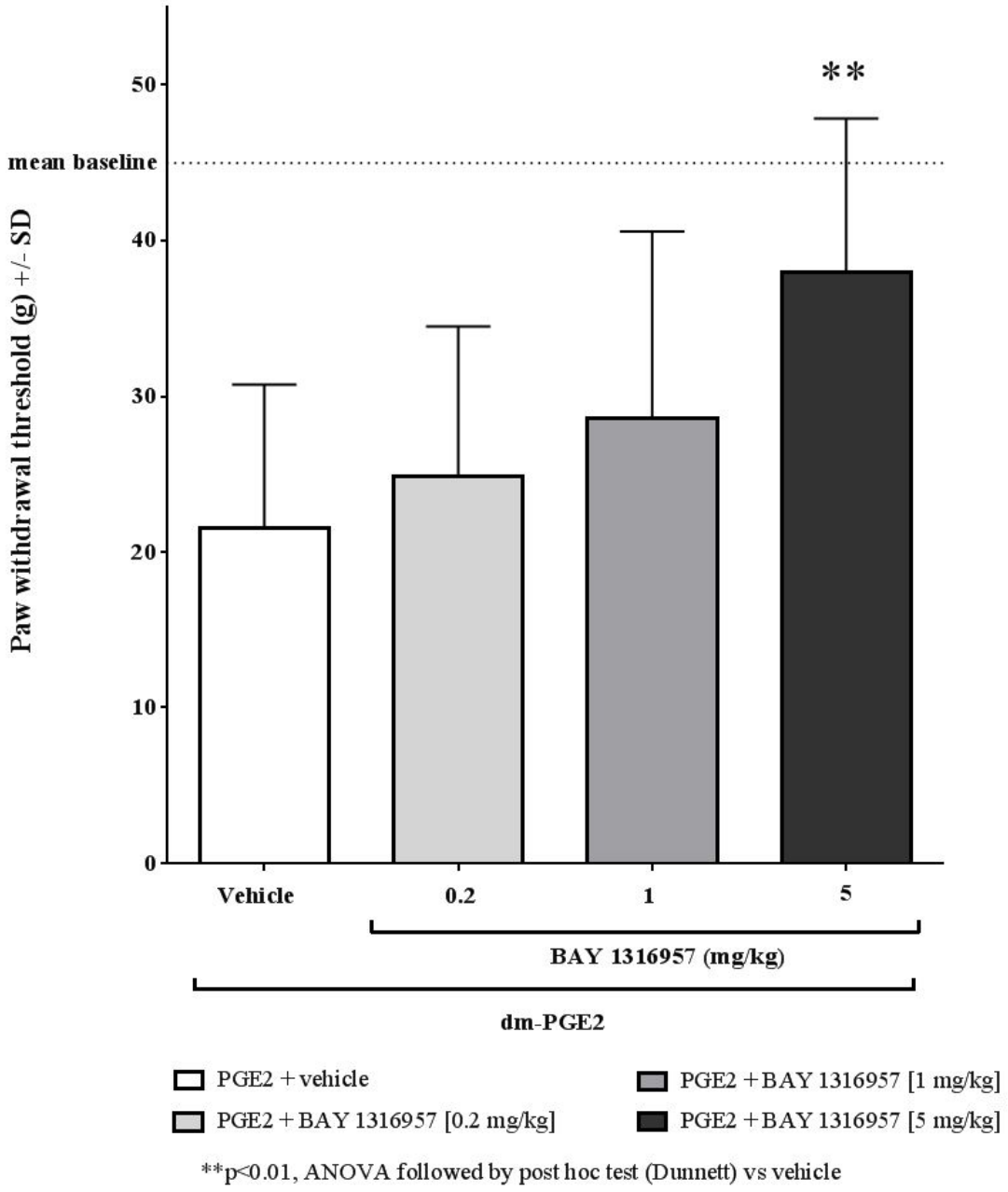
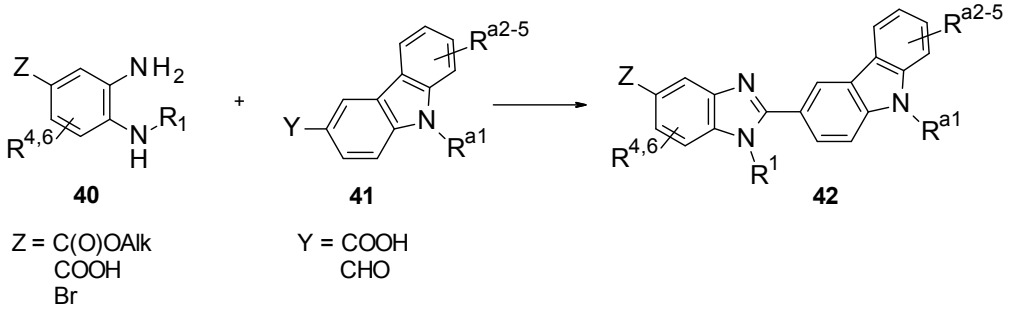


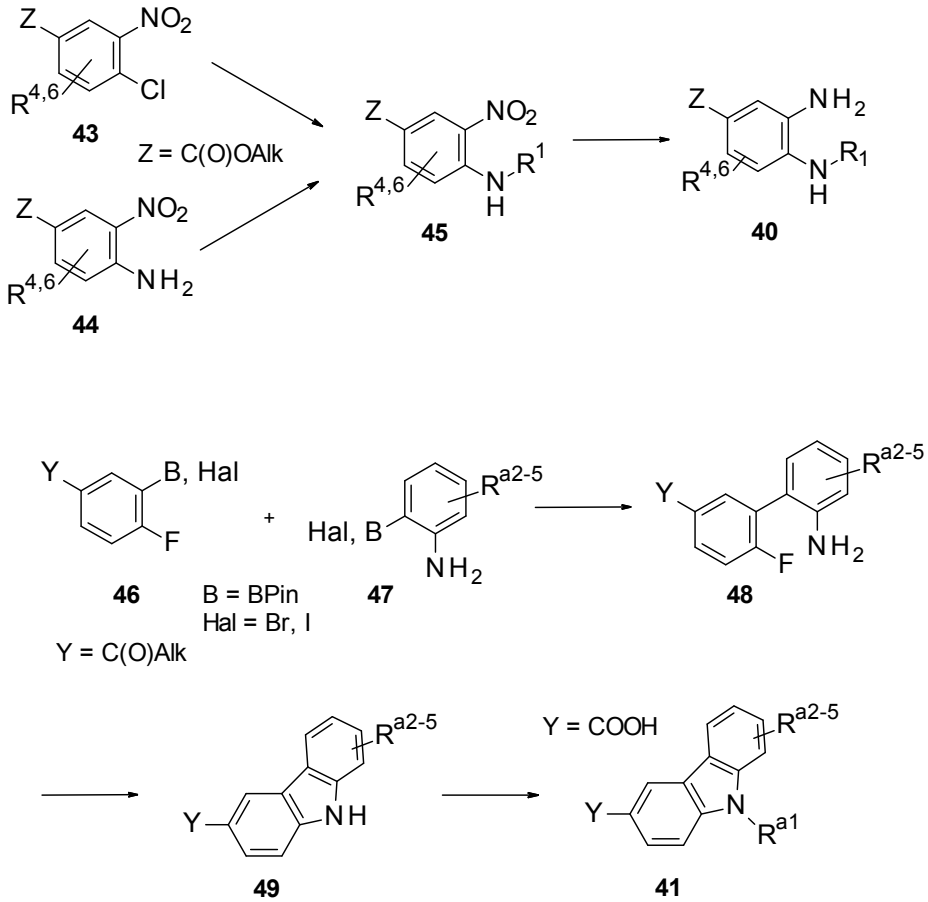
Figure 5. Oral treatment with **BAY 1316957** significantly reduced pain related paw withdrawal behavior in the rat upon mechanical stimulation of the dmPGE2 treated hind paw (Mean baseline: vehicle only treated animals)

Schemes

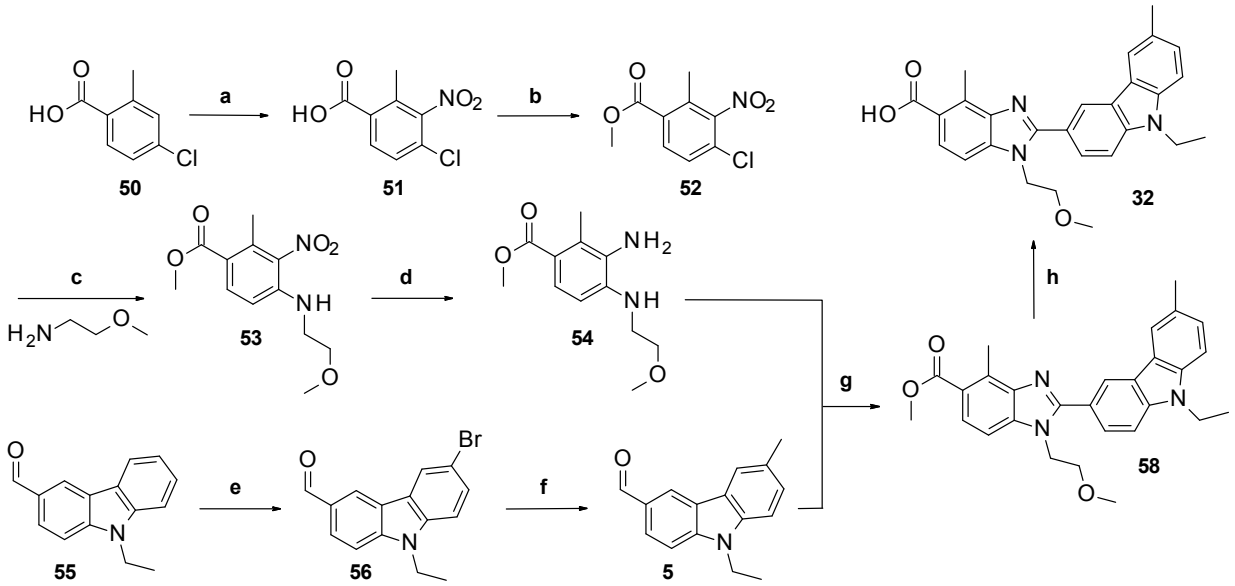
Scheme 1. General Synthetic Pathway to Benzimidazolyl Carbazole Derivatives



Scheme 2. General Synthetic Pathway to Phenylene Diamine and Carbazole Building Blocks



Scheme 3. Detailed Synthetic Pathway for Compound 32



Reagents and conditions: (a) HNO_3, quant. (1:3 mixture of 3-NO_2/ 5-NO_2 regiosiomers); (b) MeOH, H_2SO_4, 95%; (c) DMSO, 80°C, followed by chromatographic separation of 3-NO_2/ 5-NO_2 regiosiomers; (d) H_2, Pd/C, MeOH, THF, quant; (e) NBS, 76%; (f) $MeB(OH)_2$ [Pd], 79%; (g) $Na_2S_2O_5$, 89%.; (h) LiOH/ H_2O/ dioxane, 87%.

ASSOCIATED CONTENT

Supporting Information.

The following supporting information is available free of charge on the ACS Publications Website at DOI: NNNNN.

Identification of SMOL hEP4-R inhibitors. Summary of the results of high throughput screen (HTS) and hit to lead activities.

Synthesis of compounds **18** - **25**

Characteristics of Lead Compound **13**. A tabular summary of the essential properties of lead compound **13** (i.e. selected molecular-/physicochemical-, *in vitro* pharmacology-, safety- and pharmacokinetic characteristics).

BAY 1316957 candidate characteristics. A tabular summary of the overall profile of the clinical candidate BAY 1316957 (**32**) (again, selected molecular-/physicochemical-, *in vitro* pharmacology-, safety- and pharmacokinetic characteristics are given).

Molecular Formular Strings

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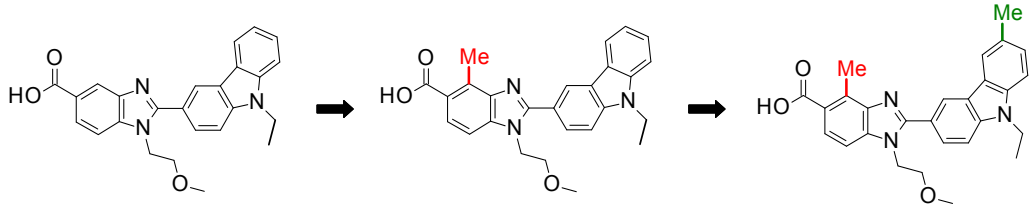
Table of Contents Graphic



	Lead Structure (**13**)	Advanced Compound (**28**)	Final Candidate (**32**)
hEP4-R	4.7 nM	25 nM	15 nM
F (rat)	48 %	92 %	90 %