Bioorganic & Medicinal Chemistry 22 (2014) 1077–1088

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Carbamazepine derivatives with P2X4 receptor-blocking activity

Maoqun Tian, Aliaa Abdelrahman, Stephanie Weinhausen, Sonja Hinz, Stefanie Weyer, Stefan Dosa, Ali El-Tayeb [†], Christa E. Müller [*]

*PharmaCenter Bonn, Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany*

ARTICLE INFO

*Article history:*
Received 15 November 2013
Revised 15 December 2013
Accepted 16 December 2013
Available online 25 December 2013

*Keywords:*
Heterocycles
Inflammation
Ligand-gated ion channel
Negative allosteric modulator
Neuropathic pain
P2X4 receptor
Structure–activity relationship
Synthesis
Tricyclics

ABSTRACT

Antagonists for the P2 receptor subtype P2X4, an ATP-activated cation channel receptor, have potential as novel drugs for the treatment of neuropathic pain and other inflammatory diseases. In the present study, a series of 47 carbamazepine derivatives including 32 novel compounds were designed, synthesized, and evaluated as P2X4 receptor antagonists. Their potency to inhibit ATP-induced calcium influx in 1321N1 astrocytoma cells stably transfected with the human P2X4 receptor was determined. Additionally, species selectivity (human, rat, mouse) and receptor subtype selectivity (P2X4 vs P2X1, 2, 3, 7) were investigated for selected derivatives. The most potent compound of the present series, which exhibited an allosteric mechanism of P2X4 inhibition, was *N*,*N*-diisopropyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (**34**, $IC_{50}$ of 3.44 μM). The present study extends the so far very limited knowledge on structure–activity relationships of P2X4 receptor antagonists.



## 1. Introduction

Purinergic receptors, which were first defined in 1976 by Burnstock, are cell-surface proteins activated by ATP and its metabolites.[1,2] There are two major families of purinergic receptors, P1 and P2, activated by extracellular adenosine and ATP/ADP, respectively.[3,4] The P2 receptors can be further subdivided into two subgroups, ATP-gated cation channels (P2X) and G protein-coupled receptors (P2Y). Seven P2X receptor subunits (P2X1–P2X7) have been identified in mammalian cells, which form homo- or hetero-trimeric channels.[3] The recently published X-ray structures of the zebrafish P2X4 receptor represent the very first crystal structures of a P2X receptor.[5,6] A chalice-shaped trimeric structure, previously suggested by Nicke et al.[7] was confirmed. Each subunit is composed of a large rigid ectodomain, two continuous transmembrane α-helical segments and intracellular termini.[5] The crystal structure of the P2X4 receptor in complex with ATP reveals three equivalent intersubunit ATP-binding sites, which are 40 Å from the extracellular boundary of the transmembrane domain and locate at each of the three pairs of subunit interfaces in the trimeric receptor.[6] ATP binding promotes an iris-like opening of the ion channel pore, which is caused by the flexing of the lower body domain and expanding the region of the receptor proximal to the ion channel pore.[6]

P2X receptors are widely distributed throughout many tissues, and play key physiological roles in nerve transmission, pain sensation and inflammation.[3] The P2X4 subtype is expressed in the central nervous system as well as peripheral tissues, for example in microglia, in testis, colon and endothelial cells.[3] After peripheral nerve injury, microglia in the dorsal horn of the spinal cord becomes activated and upregulates the expression of the P2X4 receptors, which has been identified as an important determinant of neuropathic pain.[8–11]

P2X4 receptor knockout mice showed decreased inflammatory and neuropathic pain and reduced allodynia.[12] Therefore, P2X4 receptor antagonists are of great interest as potential new drugs for the treatment of neuropathic pain, a severe chronic condition, which is difficult to treat with current analgesic drugs. Furthermore, recent evidence has accumulated that P2X4 antagonists may also be useful for the treatment of other inflammatory diseases including diabetic nephropathy,[13] joint inflammation and arthritis,[14] spinal cord injury,[15] and the prevention of excitotoxic damages after epileptic seizures.[16]

However, only few P2X4 antagonists are known so far that allow blockade of P2X4 receptor signaling, and all of them show only moderate potency and/or are lacking selectivity.[17–19] The

* Corresponding author. Tel.: +49 228 73 2301; fax: +49 228 73 2567.
*E-mail address:* christa.mueller@uni-bonn.de (C.E. Müller).
† A. E.-T. is on leave from the University of Al-Azhar, Assiut, Egypt.

0968-0896/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2013.12.035

antidepressant drug paroxetine (**I**) represents one of the few reported noncompetitive P2X4 antagonists, which displayed an $IC_{50}$ of around 2 μM in calcium flux studies at both human and rat P2X4 receptors (Fig. 1).[20] However, a subsequent investigation by Khakh's group suggested an indirect reduction of P2X4 responses by paroxetine in a microglial cell line, which may be attributed to interfering with lysosomal trafficking and a subsequent down-regulation of P2X4 receptor expression.[21] Recently, *N*-substituted phenoxazine derivatives have been developed by our group as potent P2X4 receptor antagonists.[22] The published data represent the only structure–activity relationship (SAR) study of P2X4 antagonists reported to date. *N*-(Benzyloxycarbonyl)phenoxazine (**II**), which exhibits an $IC_{50}$ of 0.189 μM at the human P2X4 receptor, is currently one of the most potent antagonists at human P2X4 receptors.[22]

With the goal to further explore the SARs of P2X4 receptor antagonists, and considering the structural similarity of the tricyclic heterocycles of phenoxazines (**II**) and carbamazepine (**III**), we planned to design and synthesize a series of carbamazepine derivatives, and evaluate them as P2X4 receptor antagonists. Carbamazepine is a well-known anticonvulsant drug used in the treatment of epilepsy. It is also used for treating trigeminal neuralgia, as well as bipolar disorder.[23]

In the present study, a broad range of functionalities, including ureas, carbamates and amides, at the *N*5 position of the dibenzo[*b*,*f*]azepine scaffold of **III** was explored. Additionally, palladium ligand-controlled cyclization was employed to synthesize derivatives containing various substituents on the aryl rings. Further derivatives were prepared by functionalization of the 10,11-double bond and investigated for potential interactions with P2X4 receptors.

## 2. Results and discussion

### 2.1. Chemistry

#### 2.1.1. Functionalization at the *N*5 nitrogen atom of the dibenzazepine core structure

Modification of the lead structure of carbamazepine was initiated by variation of the functionalities at the *N*5-atom by preparing ureas, carbamates, and amides, respectively. Amide derivatives **2** and **3** were synthesized by the reaction of dibenz[*b*,*f*]azepine (**1**) with acid anhydrides, or acyl chlorides, respectively, in good yields (Scheme 1). Further reduction of the obtained amide with lithium aluminum hydride (LAH) gave the amine **4**.

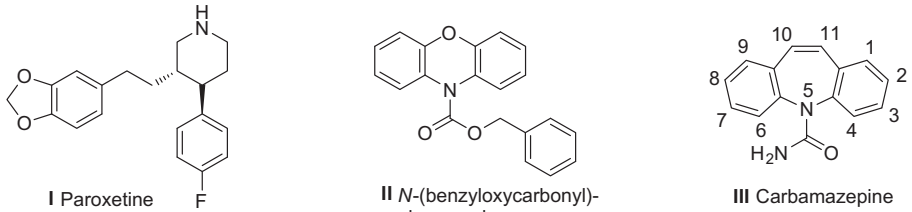
The first attempt to synthesize carbamate **5** was performed by the reaction of dibenz[*b*,*f*]azepine-5-carbonyl chloride (**8**) with methanol in the presence of triethylamine. However, only traces of the product were observed by liquid chromatography-mass spectrometry (LC–MS) analysis after the reaction had been performed under reflux for 24 h. In an alternative approach, the reaction of dibenz[*b*,*f*]azepine with the corresponding chloroformates in the absence of solvent at 90–110 °C yielded the desired carbamates **5**–**7** in moderate to good yields.

The urea derivatives **9**–**31** were synthesized by the reaction of dibenz[*b*,*f*]azepine-5-carbonyl chloride (**8**) with the corresponding primary or secondary aliphatic amines in dichloromethane at room temperature, or 40 °C, respectively, in good yields. Reactions with aromatic amines were performed at 100–110 °C to achieve reasonable reaction rates and yields.

In an attempt to synthesize urea derivative **34** with an *N*,*N*-diisopropyl substituent, no desired product was obtained in the reaction of dibenz[*b*,*f*]azepine-5-carbonyl chloride with diisopropylamine, presumably due to the significant steric hindrance of the bulky *N*,*N*-diisopropyl substituent. Notably, the reaction of dibenz[*b*,*f*]azepine with diisopropylcarbamoyl chloride in the presence of LDA gave 4-substituted dibenz[*b*,*f*]azepine derivative **32** as the main product, most likely via a dilithio intermediate, which was subsequently trapped by diisopropylcarbamoyl chloride.[24] It should be noted that no desired product, namely the urea derivative **34**, was observed under this reaction condition. The structure of **32** was confirmed by $^{1}H$ and $^{13}C$ NMR spectroscopy, and mass spectrometric data. Further reaction of **32** with triphosgene and subsequent treatment with diethylamine yielded urea derivative **33**. The substitution pattern of **33** was clearly confirmed by the nuclear Overhauser effect (NOE) between the ethyl group and *iso*-propyl group. In another approach, compound **34** was finally obtained by the reaction of dibenz[*b*,*f*]azepine with diisopropylcarbamoyl chloride in refluxing toluene.

#### 2.1.2. Introduction of substituents on the phenyl ring and replacement of phenyl by heteroaromatic rings

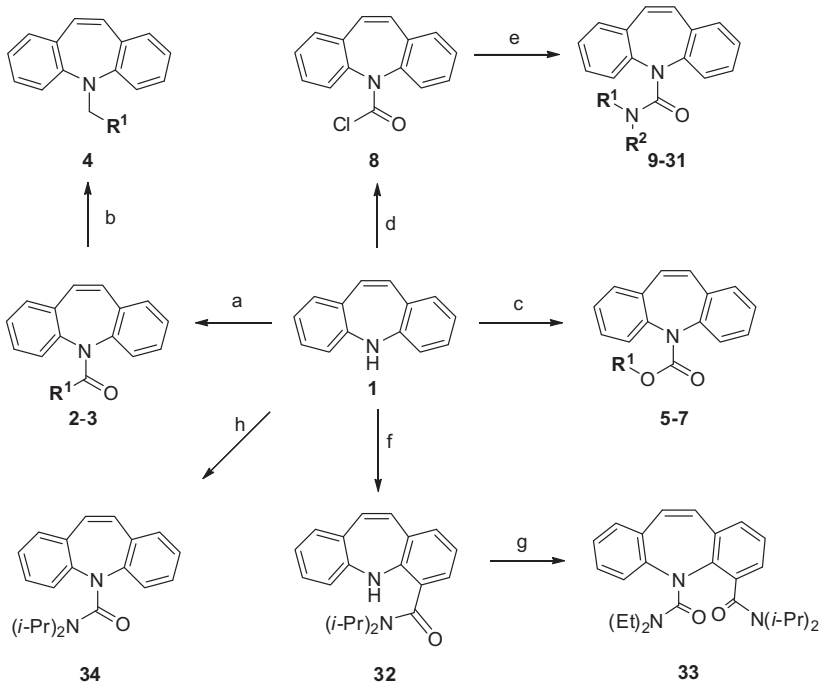
The introduction of various substituents on the phenyl rings of the dibenzazepine core structure cannot be easily achieved. The synthesis of dibenzazepine analogs usually requires harsh, functional group-intolerant conditions, for example gas-phase dehydrogenation of iminodibenzyls at high temperatures.[25] On the other hand, functionalization of the tricyclic dibenzazepine core is usually inefficient and unselective.[26] Recently, Pd-ligand controlled cyclization was developed for a general and efficient preparation of functionalized dibenzazepines.[27] Employing this method, various substituents were introduced on the phenyl ring. The palladium-catalyzed cyclization of 2-bromostyrene and substituted 2-chloroanilines was performed in refluxing 1,4-dioxane in the presence of tris(dibenzylideneacetone)dipalladium [$Pd_2(dba)_3$], 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl (DavePhos), and sodium *tert*-butoxide (NaO*t*-Bu) to provide substituted dibenzazepine derivatives in moderate yields (Scheme 2). The urea derivatives were prepared by further reaction of dibenzazepine derivatives with sodium isocyanate in good yields.[28] However, in the case of pyridobenzazepine **42**, attempts to react it with sodium isocyanate failed, presumably due to the significant electronic




human P2X4: $IC_{50}$ 1.87 μM
rat P2X4: $IC_{50}$ 2.45 μM

human P2X4: $IC_{50}$ 0.189 μM
rat P2X4: $IC_{50}$ 2.10 μM

**Figure 1.** Paroxetine (**I**), *N*-(benzyloxycarbonyl)phenoxazine (**II**) and carbamazepine (**III**).




| cpd. | $R^1$ | $R^2$ |
|---|---|---|
| **2** | methyl | - |
| **3** | phenyl | - |
| **4** | phenyl | - |
| **5** | methyl | - |
| **6** | *i*-butyl | - |
| **7** | benzyl | - |
| **9** | ethyl | ethyl |
| **10** | benzyl | H |
| **11** | methyl | H |
| **12** | $-CH_2CH_2OCH_2CH_2-$ | |
| **13** | dimethylaminopropyl | H |
| **14** | *i*-propyl | H |
| **15** | 4-methoxyphenylethyl | H |
| **16** | cyclohexyl | H |
| **17** | phenylethyl | H |

| cpd. | $R^1$ | $R^2$ |
|---|---|---|
| **18** | $-CH_2CH_2CH_2CH_2CH_2-$ | |
| **19** | $-CH_2CH_2CH_2CH_2-$ | |
| **20** | mehyl | methyl |
| **21** | butyl | H |
| **22** | propyl | propyl |
| **23** | dimethylaminoethyl | methyl |
| **24** | *t*-butyl | H |
| **25** | 1-methylpiperidin-4-yl | H |
| **26** | hydroxyethyl | hydroxyethyl |
| **27** | $-CH_2CH_2CH(OH)CH_2CH_2-$ | |
| **28** | 4-hydroxycyclohexyl | H |
| **29** | 4-aminocyclohexyl | H |
| **30** | phenyl | H |
| **31** | pyridin-4-yl | H |

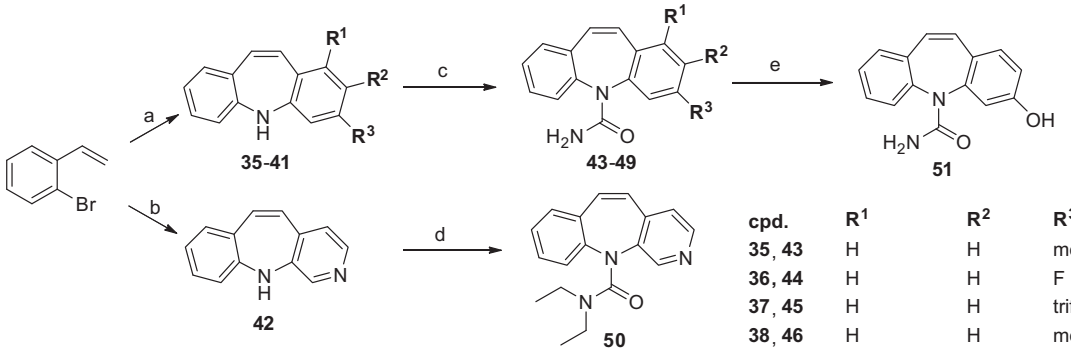
**Scheme 1.** Substitution of dibenz[*b*,*f*]azepine (**1**) at *N*5: synthesis of amides, carbamates, ureas and amines. Reagents and conditions: (a) anhydride or acyl chloride, rt to 100 °C, 3 h, yields 71–81%; (b) lithium aluminum hydride (LAH), tetrahydrofuran, (THF), rt, 1 h, yield 26%; (c) chloroformates, 90–110 °C, 3 h, yields 57–91%; (d) triphosgene, dichloromethane (DCM), 0 °C to rt; (e) $R^1R^2NH$, rt to 110 °C, dichloromethane or toluene, 1–4 h, yields 50–95%; (f) lithium diisopropylamide (LDA), diisopropylcarbamoyl chloride, THF, yield 56%; (g) triphosgene, diethylamine, DCM, yield 76%; (h) diisopropylcarbamoyl chloride, toluene, reflux, yield 55%.

effects of the pyridine ring, which decreases the nucleophilicity of the *N*5 atom. Gratifyingly, the urea derivative **50** could be obtained by the reaction of **42** with diethylamine in the presence of lithium bis(trimethylsilyl)amide (LHMDS). One of the metabolites of carbamazepine, namely 3-hydroxy-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (**51**), was prepared by demethylation of the 3-methoxy-substituted precursor **46** with boron tribromide in 37% yield.[29]

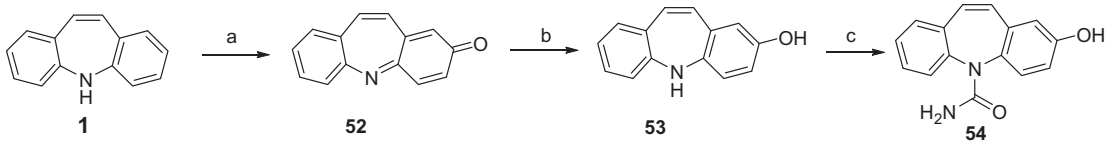
A starting material 4-*O*-substituted 2-chloroaniline is not readily available. Therefore, 2-hydroxy-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (**54**), which is another metabolite of carbamazepine, was synthesized by an alternative approach (Scheme 3). Oxidation of **1** with Fremy's salt followed by the reduction of **52** with sodium dithionite gave 2-hydroxydibenzazepine **53** in moderate yield.[30] Reaction of **53** with sodium isocyanate and subsequent selective hydrolysis of the intermediate with sodium hydrogencarbonate yielded 2-hydroycarbamazepine **54**. It should be noted that stronger bases for example sodium carbonate or sodium hydroxide resulted in extensive hydrolysis of the urea functionality to regenerate **53**.

### 2.1.3. Functionalization at positions 10 and 11

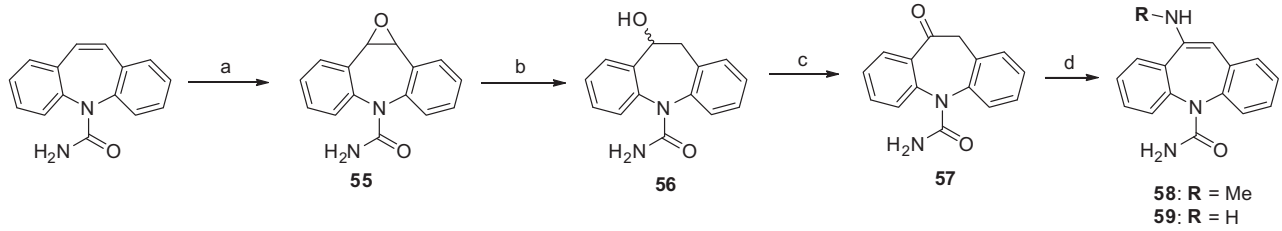
It is well known that the 10,11-double bond of carbamazepine is metabolically unstable resulting in several active metabolites.[31] In order to get a deeper insight into the structure–activity relationships of carbamazepine derivatives at P2X4 receptors, a few derivatives were to be prepared by functionalization of the 10,11-double bond (Scheme 4). The racemic alcohol **56** was synthesized by epoxidation of carbamazepine and subsequent palladium-catalyzed hydrogenation of the epoxide **55**. In another approach, oxidation of **56** gave oxcarbamazepine **57** in 90% yield. In the reaction of **57** with amines in the presence of $NaBH_3CN$, $Na(CH_3COO)_3BH$, or $Ti(OiPr)_4/NaBH_4$, which are the reagents




**Scheme 2.** Synthesis of carbamazepine derivatives with substituents on the phenyl ring and aza-analogs. Reagents and conditions: (a) 2-chloroaniline derivatives, $Pd_2(dba)_3$, DavePhos, NaO*t*-Bu, 1,4-dioxane, 115 °C, yields: 20–65%; (b) 4-chloro-3-aminopyridine, $Pd_2(dba)_3$, DavePhos, NaO*t*-Bu, 1,4-dioxane, 115 °C, yield 26%; (c) NaOCN, HOAc, 65 °C, 2 h, yields: 70–81%. (d) LHMDS, diethylamine, 0 °C to rt, 2 h, yield: 24%; (e) $BBr_3$, DCM, 0 °C to rt, 24 h, yield: 37%.




**Scheme 3.** Synthesis of 2-hydroxycarbamazepine. Reagents and conditions: (a) $(KSO_3)_2NO$, $Na_2HPO_4$, $H_2O$/acetone, rt 3 h, yield: 34%; (b) $Na_2S_2O_4$, rt, 69%; (c) NaOCN, HOAc, 70 °C, 2 h; $NaHCO_3$, $H_2O$/MeOH, 0 °C, 30 min, yield: 29% over two steps.




**Scheme 4.** Synthesis of carbamazepine derivatives by functionalization of the 10,11-double bond. Reagents and conditions: (a) HOOAc, $KMnO_4/Al_2O_3$, DCM, rt, 2 h, yield: 66%; (b) $H_2$, Pd/C, $N(Et)_3$, MeOH–$H_2O$, 2 h, yield: 63%; (c) $K_2Cr_2O_7$, $CH_3CO_3H$, yield: 90%; (d) $RNH_2$, $Ti(OiPr)_4$, EtOH.

frequently used for reductive amination reactions, the formation of the corresponding amines could not be observed. Notably, the 10-amino-substituted carbamazepine derivatives **58** and **59**, resulting from the aromatization of the imine intermediates, were isolated as the main products.

Overall, 47 carbamazepine derivatives, including 32 novel compounds, were synthesized, and their structures and purities (>95%) were firmly confirmed by $^1H$ and $^{13}C$ NMR spectroscopy, and LC–MS analysis, respectively.

## 2.2. Biological evaluation at P2X4 receptors

Inhibition of ATP-induced calcium influx in 1321N1 astrocytoma cells stably transfected with the human P2X4 receptor was determined as previously described.[22] The compounds were initially tested at a high concentration of 10–300 μM depending on their solubility. Full concentration-inhibition curves were determined for potent compounds and $IC_{50}$ values were calculated. Results are collected in Table 1.

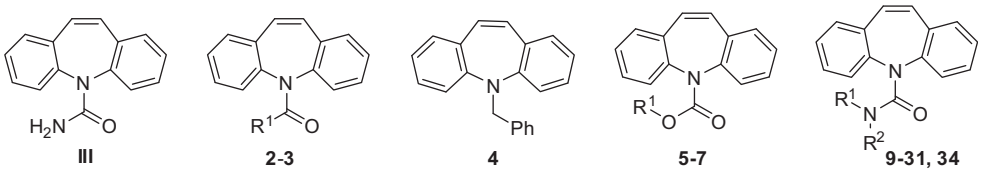
Selected compounds were additionally investigated at rat and mouse P2X4 receptors (Table 2). The most potent compounds were also evaluated for their inhibitory potency at P2X1, P2X2, P2X3, and P2X7 receptors (Table 2).

## 2.3. Structure–activity relationships of *N*5-substituted dibenzazepines

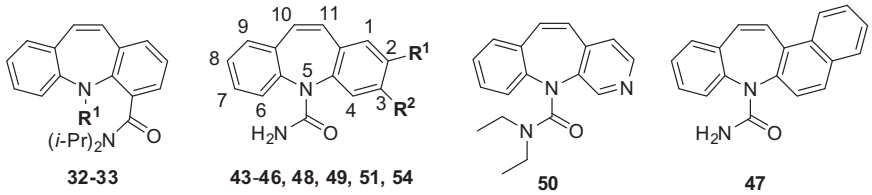
Under the experimental conditions established in our laboratory, paroxetine (**I**) exhibited P2X4-antagonistic potency with an $IC_{50}$ of 4.68 μM, which is comparable to the reported results.[20] In preliminary experiments the commercially available anticonvulsant drug carbamazepine (**III**) showed some P2X4-antagonistic activity at micromolar concentrations. Further testing revealed that it was a relatively weak P2X4 receptor antagonist at human P2X4 receptors (35% inhibition of ATP-induced calcium influx at 100 μM, see Table 1).

The synthesized carbamazepine derivatives and analogs that were modified at *N*5 of the dibenz[*b*,*f*]azepine core can be divided

**Table 1**
Potency of carbamazepine derivatives and analogs at human P2X4 receptors



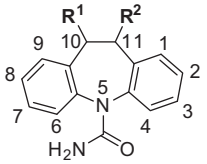

| Compound | $R^1$ | $R^2$ | Human P2X4 $IC_{50}$ ± SEM[a] (μM) |
|---|---|---|---|
| Paroxetine (**I**) | | | 4.68 ± 1.38 |
| *N*-(Benzyloxy carbonyl)-phenoxazine (**II**)[22] | | | 0.189 ± 0.059 (77%)[b] |
| Carbamazepine (**III**) | — | — | >100 (35%)[b] |
| *Amides* | | | |
| **2** | Methyl | — | >100 (39%)[b] |
| **3** | Phenyl | — | 23.3 ± 5.8 (75%)[b] |
| *Amine* | | | |
| **4** | — | — | >100 (34%)[b] |
| *Carbamates* | | | |
| **5** | Methyl | — | ca. 100 (51%)[b] |
| **6** | *sec*-Butyl | — | ca. 100 (58%)[b] |
| **7** | Benzyl | — | 6.02 ± 1.64 (73%)[c] |
| *Ureas* | | | |
| **9** | Ethyl | ethyl | 11.4 ± 1.5 (95%)[b] |
| **10** | Benzyl | H | 78.9 ± 19.6 (75%)[b] |
| **11** | Methyl | H | ⩾100 (47%)[b] |
| **12** | $-CH_2CH_2OCH_2CH_2-$ | | ⩾100 (50%)[b] |
| **13** | Dimethylaminopropyl | H | ca. 100 (54%)[b] |
| **14** | Isopropyl | H | 19.2 ± 7.3 (47%)[b] |
| **15** | 4-Methoxyphenylethyl | H | 27.4 ± 3.8 (83%)[b] |
| **16** | Cyclohexyl | H | 33.8 ± 6.9 (100%)[b] |
| **17** | 2-Phenylethyl | H | 36.2 ± 7.2 (107%)[b] |
| **18** | $-CH_2CH_2CH_2CH_2CH_2-$ | | 13.7 ± 2.8 (72%)[b] |
| **19** | $-CH_2CH_2CH_2CH_2-$ | | 34.1 ± 2.1 (98%)[b] |
| **20** | Methyl | methyl | ⩾100 (49%)[b] |
| **21** | Butyl | H | ⩾100 (49%)[b] |
| **22** | Propyl | Propyl | 7.78 ± 1.60 (49%)[c] |
| **23** | Dimethylaminoethyl | Methyl | ⩾100 (42%)[b] |
| **24** | *t*-Butyl | H | 13.8 ± 1.6 (92%)[b] |
| **25** | 1-Methylpiperidin-4-yl | H | ⩾100 (56%)[b] |
| **26** | Hydroxyethyl | Hydroxyethyl | >>100 (2%)[b] |
| **27** | $-CH_2CH_2CH(OH)CH_2CH_2-$ | | >>100 (−18%)[b] |
| **28** | 4-Hydroxycyclohexyl | H | >>20 (0%)[d] |
| **29** | 4-Aminocyclohexyl | H | >>10 (11%)[c] |
| **30** | Phenyl | H | ca. 100 (49%)[b] |
| **31** | Pyridin-4-yl | H | >100 (30%)[b] |
| **34** | *i*-Propyl | *i*-Propyl | 3.44 ± 1.15 (71%)[c] |



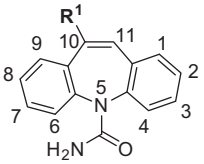

| Compound | $R^1$ | $R^2$ | Human P2X4 $IC_{50}$ ± SEM (μM)[a] |
|---|---|---|---|
| **32** | H | — | 30.6 ± 13.1 (74%)[b] |
| **33** | Diethylcarbamoyl | — | 52.5 ± 16.3[b] |
| **43** | H | Methyl | >100 (21%)[b] |
| **44** | H | F | >>100 (−2%)[b] |
| **45** | H | $CF_3$ | 54.6 ± 13.7 (72%)[b] |
| **46** | H | OMe | >100 (30%)[b] |
| **47** | — | — | 51.4 ± 11.4 (91%)[b] |
| **48** | H | Dimethylamino | 15.3 ± 5.9 (39%)[b] |
| **49** | F | H | >100 (11%)[b] |
| **50** | — | — | ca. 100 (52%)[b] |
| **51** | H | OH | 72.8 ± 13.2 (99%)[e] |
| **54** | OH | H | 146 ± 19 (82%)[e] |

(*continued on next page*)

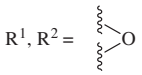
**Table 1** (*continued*)




**55, 56, 60-62**




**57-59, 63**

| Compound | $R^1$ | $R^2$ | Human P2X4 $IC_{50}$ ± SEM[a] (μM) |
|---|---|---|---|
| **55** | $R^1, R^2$ =  | | >>10 (1%)[c] |
| **56** | *rac*-OH | H | >100 (28%)[b] |
| **60**[f] | H | H | >100 (35%)[b] |
| **61**[g] | (*S*)-Acetoxy | H | >20 (25%)[d] |
| **62**[f] | *rac*-Ethoxy | H | >100 (17%)[b] |
| **57** | OH | — | >10 (10%)[c] |
| **58** | MeNH | — | 36.9 ± 8.7 (80%)[b] |
| **59** | $NH_2$ | — | >10 (12%)[c] |
| **63**[f] | Methoxy | — | >100 (34%)[b] |

[a] $n = 3$.
[b] Percent inhibition at 100 μM of test compound.
[c] Percent inhibition at 10 μM of test compound.
[d] Percent inhibition at 20 μM of test compound.
[e] Percent inhibition at 300 μM of test compound.
[f] Purchased from Sigma–Aldrich.
[g] Extracted from commercially available eslicarbazepine acetate 800 mg tablets (Zebinix®).

**Table 2**
Potency of selected carbamazepine derivatives at P2X4 orthologues and different P2X receptor subtypes

| Compd. | Human P2X4 | Mouse P2X4 | Rat P2X4 | Human P2X1 | Human P2X2 | Human P2X3 | Human P2X7 |
|---|---|---|---|---|---|---|---|
| **9** | 11.4 ± 1.5 | >100 (27%)[a] | 43.0 ± 10.8 | 25.2 ± 3.0 | >100 (1%)[a] | 14.2 ± 0.9 | 69.8 ± 12.6 |
| **16** | 33.8 ± 6.9 | 98.4 ± 12.6 | 109 ± 2 | 69.6 ± 12.1 | >100 (11%)[a] | 28.0 ± 3.9 | 40.8 ± 5.8 |
| **18** | 13.7 ± 2.8 | 102 ± 11 | 95.8 ± 9.8 | 20.7 ± 1.9 | >100 (4%)[a] | 7.98 ± 1.22 | >100 (−10%)[a] |
| **19** | 34.1 ± 2.1 | 123 ± 18 | 69.3 ± 17.4 | 68.8 ± 14.5 | >100 (6%)[a] | 29.8 ± 4.3 | >100 (33%)[a] |
| **22** | 7.78 ± 1.60 | >100 (38%)[a] | 35.9 ± 4.6 | 4.55 ± 1.23 | >100 (−2%)[a] | 15.2 ± 0.5 | >100 (−11%)[a] |
| **34** | 3.44 ± 1.15 | 14.9 ± 2.2 | 54.6 ± 13.9 | 5.32 ± 1.91 | >100 (8%)[a] | 7.18 ± 2.86 | 95.2 ± 8.3 |

[a] Inhibition of calcium flux at 100 μM concentration. $IC_{50}$ ± SEM (μM).

into four subgroups (Table 1): amide (**2**, **3**), amine (**4**), carbaminic acid (**5**–**7**), and urea (**9**–**31** and **34**) derivatives. Methylamide **2** showed similarly low activity as carbamazepine, while the phenylamide **3** exhibited somewhat higher P2X4-antagonistic activity ($IC_{50}$ of 23 μM). However, the benzyl-substituted tertiary amine **4** showed lower activity, and therefore no further amine derivatives were explored. A carbonyl group attached to the ring nitrogen atom appeared to be required. Subsequently, three carbamate derivatives were investigated (**5**–**7**). The alkyl carbamates **5** and **6** showed weak activity (estimated $IC_{50}$ value around 100 μM), whereas the benzyl carbamate **7** displayed significantly higher potency with an $IC_{50}$ of 6.02 μM. A structurally related phenoxazine derivative had recently been developed as a potent P2X4 receptor antagonist.[22]

As a next step a series of urea derivatives was explored. Investigation of monoalkyl-substituted urea derivatives showed that small, branched substituents were best tolerated, namely the isopropyl derivative **14** (19.2 μM) and the *tert*-butyl derivative **24** (13.8 μM), while smaller (methyl derivative **11**) and linear alkyl substituents (butyl derivative **21**) resulted in very weakly active compounds ($IC_{50}$ ca. 100 μM). The cyclohexyl derivative **16** was intermediate in potency ($IC_{50}$ 33.8 μM). Polar substituents on the alkyl substituent (compounds **13**, **25**, **28**, **29**) were not well tolerated. Phenyl-substitution (**30**) or bioisosteric substitution with a 4-pyridyl residue (**31**) were also not tolerated. However, phenyl residues connected to the urea via a methylene (benzyl derivative **10**, $IC_{50}$ 78.9 μM) or even more so by an ethylene linker (phenethyl derivative **17**, $IC_{50}$ 36.2 μM; and *p*-methoxyphenethyl derivative **15**, $IC_{50}$ 27.4 μM) led to somewhat higher potency.

Disubstitution of the urea $NH_2$ function resulted in increased potency. The rank order of potency of dialkyl-substituted urea derivatives was: diisopropyl (**34**, $IC_{50}$ 3.44 μM) > dipropyl (**22**, $IC_{50}$ 7.78 μM) > diethyl (**9**, $IC_{50}$ 11.4 μM > dimethyl (**20**, $IC_{50}$ ca. 100 μM) = *N*-methyl-*N*-dimethylaminoethyl (**23**, ca. 100 μM) > dihydroxyethyl (**26**, >> 100 μM). We also investigated sterically fixed derivatives, in which both *N*-substituents were integrated into a cyclic structure (compounds **12**, **18**, **19**, **27**). The rank order of potency was as follows: piperidine (**18**, $IC_{50}$ 13.7 μM) > pyrrolidine (**19**, $IC_{50}$ 34.1 μM) > morpholine (**12**, $IC_{50}$ ca. 100 μM) >> *p*-hydroxypiperidine (**27**, >> 100 μM). The SARs observed for the *N*,*N*-disubstituted urea derivatives confirmed the trend observed with monosubstituted compounds: small, branched alkyl substituents were optimal while polar residues (e.g., in **12**, **27**) were not tolerated at that position. The diisopropylurea derivative **34** was the best compound of the present series.

### 2.4. Structure–activity relationships of ring-modified carbamazepine derivatives and analogs

Annelation of another benzene ring at the 1,2-position of the dibenzazepine (compound **47**) slightly increased potency of carbamazepine resulting in a moderately potent compound (51.4 μM). In the 2-position of the dibenzazepine ring only F and OH were tested, both substituents resulting in inactive or very weakly active compounds (**49**, **54**). Introduction of a nitrogen atom at the 3-position of the dibenzazepine ring to obtain aza-analog **50** resulted in a large drop in potency (compare **9** and **50**). A hydroxyl residue in the 3-position was well accepted (compound **51**). Substitution in position 4 of the dibenzazepine ring with a large diisopropylaminocarbonyl residue was tolerated (**32**, **33**; compare **32** with carbamazepine). Substitution at the 3-position of carbamazepine with fluoro (**44**), methyl (**43**), or methoxy (**46**) did not improve the potency of the lead structure and resulted in virtually inactive compounds. Hydroxy-(**51**, 72.8 μM), trifluoromethyl-(**45**, 54.6 μM), and, even more so, dimethylamino-substitution (**48**, 15.3 μM) increased the potency of carbamazepine. Substitution at the 10-position of the dibenzazepine core of carbamazepine with a hydroxy (**57**), methoxy (**63**) or amino (**59**) residue did not show positive effects on potency. However, methylamino substitution led to a moderately potent P2X4 receptor antagonist (**58**, 36.9 μM). Carbamazepine derivatives, in which the 10,11-double bond of the dibenzazepine ring system was hydrogenated and the resulting dihydrocarbamazepine (**60**) was substituted with a 10,11-epoxy ring (**55**), an ethoxy (**62**) or an (*S*)-acetoxy residue (**61**) were investigated. The latter compound corresponds to the marketed antiepileptic prodrug eslicarbazepine acetate, which is converted in vivo by ester hydrolysis to the corresponding alcohol. All of these hydrogenated carbamazepine derivatives were inactive.

In conclusion, polar substituents appear to be well tolerated at the dibenzazepine tricycle especially in positions 3, 4 and 10. The best modification of the present series was the 3-dimethylamino-substituted carbamazepine **48** (15.3 μM). Substitution of the dibenzazepine tricycle should be further explored. Since polar substituents are tolerated in various positions of the ring system, such modifications could not only enhance potency but may also lead to increased water-solubility.

### 2.5. Inhibitory potency at P2X4 receptor orthologs and other P2X receptor subtypes

In order to assess whether the identified P2X4 receptor antagonists are not only active at human, but also at the rat and mouse receptor orthologs, selected potent compounds were additionally tested in calcium flux assays at rat and mouse P2X4 receptors (Table 2).

All of the selected compounds exhibited the highest potencies at the human P2X4 receptor. Compounds **16**, **18**, **19**, **22** and **34** showed a slight (3–7 fold) drop in potency at the mouse P2X4 receptor compared with the human P2X4 receptor, whereas diethyl- and dipropyl-substituted urea derivatives **9** and **22** were virtually inactive at the mouse P2X4 receptor. On the other hand, compounds **9** and **22** exhibited only a 4–5 fold drop in potency at the rat P2X4 receptor compared with the human orthologue. Compounds **16**, **18** and **19** were nearly equipotent at rat and mouse P2X4-receptors. The bulky diisopropyl-substituted urea **34** was 10-fold less active at the rat P2X4 receptor than at the human orthologue. Overall, it is demonstrated that significant differences between potencies at human versus rodent P2X4 receptors may be a common phenomenon. A similar result had been observed for the previously reported *N*-substituted phenoxazine derivatives with P2X4 receptor antagonistic activity.[22]

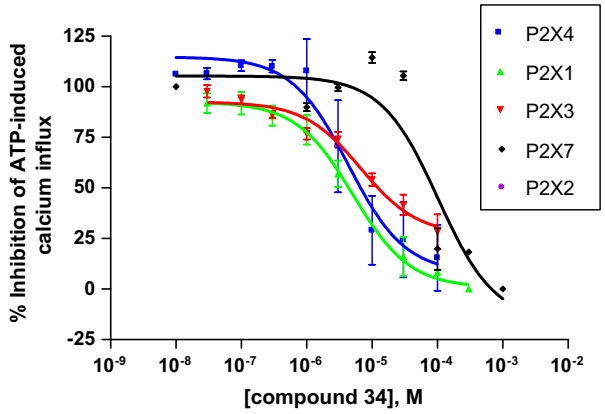



**Figure 2.** Inhibition of ATP-induced calcium influx by compound **34** at different P2X receptor subtypes. ATP concentrations correspond to the determined $EC_{80}$ values. The corresponding $IC_{50}$ values are shown in Table 2.

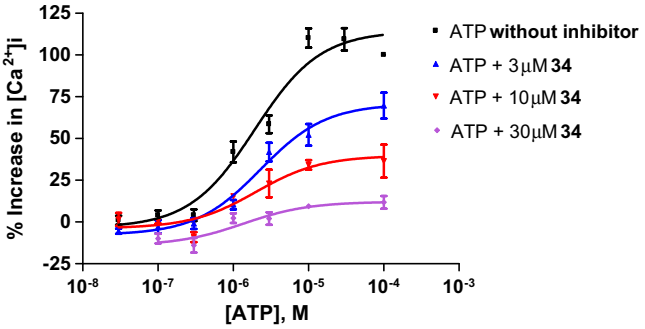



**Figure 3.** ATP-induced intracellular calcium levels in 1321N1 astrocytoma cells stably transfected with the human P2X4 receptors, measured in the absence and presence of different concentrations of compound **34**. Data were normalized to ATP without inhibitor (100%) and buffer (0%). The corresponding $EC_{50}$ values and maximal effects are shown in Table 3.

**Table 3**
$EC_{50}$ values and maximal effects for ATP in the absence and presence of different concentrations of compound **34**

| ATP dose–response curve | $EC_{50}$ (μM) | $E_{max}$ (%) |
|---|---|---|
| Without inhibitor | 1.93 ± 0.45 | 100 |
| 3 μM Compound **34** | 2.18 ± 0.70 | 70 ± 17 |
| 10 μM Compound **34** | 3.47 ± 1.74 | 36 ± 14 |
| 30 μM Compound **34** | 1.78 ± 0.91 | 12 ± 5 |

Finally, selected P2X4 antagonists were investigated for their potency to inhibit the related P2X receptor subtypes P2X1, P2X2, P2X3 and P2X4. The inhibition of ATP-induced calcium influx by one representative compound **34** is shown in Figure 2. None of the compounds showed antagonistic activity at the human P2X2 receptor (Table 2). However, all of the selected compounds were able to block some of the other P2X receptor subtypes in addition to P2X4. The most potent compounds of the present series, **18**, **22** and **34**, were about equipotent at the human P2X1, P2X3, and P2X4 receptors, but selective versus P2X2 and P2X7. Our results indicate that improved P2X4 selectivity may be achieved by further optimization, since it has already been obtained versus some of the P2X receptor subtypes. On the other hand, antagonists blocking more than one P2X receptor subtype may be advantageous, since several P2X receptor subtypes are known to be involved in pain and inflammation.[32]

### 2.6. Mechanism of action

In order to assess whether the P2X4 receptor inhibition by the new antagonists is via a competitive mechanism, that is, blockade of the ATP binding site, or via an allosteric mechanism, concentration–response curves for ATP were determined in the absence and in the presence of different concentrations (3, 10, and 30 μM) of the selected, potent antagonist **34**. As shown in Figure 3 and Table 3, the concentration–response curves showed the characteristic behaviour of a negative allosteric modulator, that is, depression of the maximal response with essentially no alteration of the $EC_{50}$ values.[33,34] This result, is comparable to the previously reported data obtained with structurally related phenoxazine derivatives, which had also been identified as noncompetitive P2X4 receptor antagonists.[22]

In contrast to competitive antagonists, negative allosteric modulators may not lead to a complete blockade of the signal. In fact, we observed that some of the potent antagonists only led to a partial inhibition of ATP-mediated calcium influx even at high concentrations. For example, antagonist **48** ($EC_{50}$ 15.3 μM) showed only 39% maximal inhibition at a high concentration of 100 μM at which a maximal effect should be observed.

## 3. Conclusions

In conclusion, we have synthesized a series of mostly new carbamazepine derivatives, whose structural modification was systematically and extensively explored. These compounds were evaluated and optimized as P2X4 receptor antagonists. The most potent compound of the present series was *N*,*N*-diisopropyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (**34**) with an $IC_{50}$ of 3.44 μM at human P2X4 receptors. However, it should be noted that compound **34** is not completely P2X4-selective, since it also shows considerable potency at the P2X1 receptor subtype ($IC_{50}$ = 5.32 μM). It was found to exhibit an allosteric mechanism of action. This study represents one of the very few reported SAR studies of P2X4 receptor antagonists, which is complementary to the recent discovery of structurally related *N*-substituted phenoxazine derivatives as P2X4 receptor antagonists.[22] Further improvement of affinity and selectivity as well as physicochemical properties will be required to obtain compounds useful as pharmacological tools for in vivo studies.

## 4. Experimental section

### 4.1. General

All commercially available materials were used as purchased without further purification. Carbamazepine (**III**), compounds **60**, **62** and **63** were purchased from Sigma–Aldrich. Compound **61** was extracted from eslicarbazepine acetate 800 mg tablets (Zebinix®), which were purchased from a German pharmacy. If not stated otherwise, deionized water was used for the experiments. Thin-layer chromatography (TLC) was performed using TLC aluminum sheets with silica gel 60 $F_{254}$ (Merck, Darmstadt, Germany). The compounds were visualized under UV light (254 nm). Column chromatography was carried out with silica gel 0.060–0.200 mm, pore diameter ca. 6 nm. $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR) data were collected on a Bruker Avance 500 MHz NMR spectrometer at 500 ($^1H$) or 125 MHz ($^{13}C$), respectively. DMSO-$d_6$ or $CDCl_3$ was used as a solvent. Chemical shifts are reported in parts per million (ppm) relative to the deuterated solvent, that is, DMSO, $\delta$ $^1H$: 2.50 ppm, $^{13}C$: 39.5 ppm; $CDCl_3$, $\delta$ $^1H$: 7.26 ppm, $^{13}C$: 77.2 ppm; coupling constants J are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad).

The purities of the isolated final products were determined by a liquid chromatography system coupled to electrospray ionization (ESI) mass spectrometry (LC–MS) on an Applied Biosystems API 2000 LCMS/MS, HPLC Agilent 1100 system using a Phenomenex Luna 3 μ C18 column (50 × 2.00 mm). UV absorption was detected from 200 to 950 nm using a diode array detector. The purity of the compounds was determined at 220–400 nm. The purity of the compounds was proven to be ⩾95%. Compounds **III**, **60**–**63** were purchased from Sigma–Aldrich. For identification of the synthesized known compounds **2**,[26] **3**,[35] **4**,[36] **5**,[37] **9**,[38] **10**,[39] **11**,[40] **19**,[41] **30**,[42] **35**–**37**,[27] **38**,[43] **39**,[44] **41**,[45] **42**,[27] **49**,[46] **51**,[46] **52-53**,[30] **54**,[47] and **55**–**57**,[48] the analytical data were compared with literature data and provided as the Supplementary data.

### 4.2. General procedure for the synthesis of amide derivatives 2 and 3 (GP-1)

A solution of dibenzazepine (193 mg, 1.00 mmol), the appropriate acid chloride (1.2 mmol), and triethylamine (101 mg, 1.0 mmol) in dry dichloromethane (10 mL) were stirred under reflux for 3 h (TLC control). After the mixture had been cooled down to rt, dichloromethane (150 mL) was added. The mixture was washed with an aqueous saturated solution of $NaHCO_3$ (2 × 50 mL), HCl (1%, 100 mL), and subsequently with brine (100 mL). The organic layer was separated and dried with $MgSO_4$. The solvent was evaporated in vacuo. The residue was submitted to column chromatography ($SiO_2$, eluent: petroleum ether/EtOAc, 5:1) to give the desired products. An analytically pure sample was obtained by recrystallization from EtOH.

### 4.3. General procedure for the synthesis of carbamate derivatives 5–7 (GP-2)

A reaction mixture of dibenzazepine (193 mg, 1.00 mmol) and the appropriate chloroformate (2 mL) was stirred under a $N_2$ atmosphere at 100–110 °C for 3 h. After it had been cooled down to rt, dichloromethane (50 mL) was added. An aqueous solution of $NaHCO_3$ (5%, 10 mL) was added to the mixture at 0 °C. It was stirred at rt for 1 h, washed with water (150 mL) and subsequently with brine (150 mL). The organic phase was separated and dried with $MgSO_4$. The volatile components were evaporated in vacuo. Pure products were obtained by crystallization of the residues from the appropriate solvent.

### 4.4. General procedure for the synthesis of urea derivatives 9–29 (GP-3)

The corresponding aliphatic amine (2.00 mmol) was added dropwise to a solution of dibenz[*b*,*f*]azepine-5-carbonyl chloride (256 mg, 1.00 mmol) in dichloromethane (10 mL) at 0 °C. The reaction mixture was stirred at rt for 4 h. Dichloromethane (100 mL) was added to the reaction mixture. It was washed with HCl (1%, 100 mL), an aqueous solution of $NaHCO_3$ (5%, 100 mL), and subsequently with brine (100 mL). The organic phase was dried over $MgSO_4$. After evaporation of the solvent, the product was obtained by recrystallization of the residue from appropriate solvents.

### 4.5. General procedure for the synthesis of urea derivatives 30 and 31 (GP-4)

A solution of the appropriate aromatic amine (2.00 mmol) and dibenz[*b*,*f*]azepine-5-carbonyl chloride (256 mg, 1.00 mmol) in toluene (10 mL) was stirred under reflux for 2 h. After it had been cooled down to rt, the volatile components were evaporated in vacuo. The product was purified by column chromatography (silica gel, eluent DCM/MeOH 20:1). After evaporation of the eluent, an

analytically pure sample was obtained by recrystallization from EtOAc.

### 4.6. General procedure for palladium-catalyzed synthesis of dibenzazepine derivatives 35–41 (GP-5)

A mixture of 2-bromostyrene (549 mg, 3.00 mmol), the appropriate 2-chloroaniline derivative (3.00 mmol), $Pd(dba)_3$ (68 mg, 0.075 mmol), DavePhos (79 mg, 0.20 mmol), and NaO*t*Bu (864 mg, 9.00 mmol) in dry 1,4-dioxane (10 mL) was stirred under a $N_2$ atmosphere at 115 °C for 6 h. After it had been cooled down to rt, EtOAc (150 mL) was added. The mixture was washed with water ($5 \times 150$ mL) and subsequently with brine (150 mL). The organic phase was separated and dried over $MgSO_4$. After evaporation of the solvent, the residue was submitted to column chromatography ($SiO_2$, eluent: petroleum ether/EtOAc 20:1). The yellow fraction was collected, and evaporation of the eluent in vacuo gave the desired product.

### 4.7. General procedure for synthesis of urea derivatives 43–49 (GP-6)

NaOCN (98 mg, 1.5 mmol) was added in small portions to a solution of the appropriate azepine derivative (1.00 mmol) in HOAc (10 mL) at 70 °C. The reaction mixture was stirred at this temperature for 2 h. After it had been cooled down to rt, the solvent was evaporated in vacuo. Water was added to the residue. The resulting colorless solid was collected and dried. The product was obtained by recrystallization of the solid from appropriate solvents.

### 4.8. General procedure for the synthesis of 10-aminocarbamazepine derivatives 58 and 59 (GP-7)

A reaction mixture of **57** (252 mg, 1.00 mmol), titanium(IV) isopropoxide (0.6 mL, 2.00 mmol) and the appropriate amine (5.00 mmol) in absolute ethanol (10 mL) was stirred under an argon atmosphere for 6 h. Sodium borohydride (60.0 mg, 1.50 mmol) was then added and the resulting mixture was stirred at rt for 3 additional hours. The reaction was then quenched by pouring it into an aq. ammonium hydroxide solution (2 M, 2.5 mL). The mixture was extracted with DCM ($2 \times 50$ mL). The combined organic phase was washed with brine, and dried with $MgSO_4$. The solvent was evaporated in vacuo. The product was obtained by recrystallization of the residue from ethyl acetate.

### 4.9. *N*-Isobutyl-5*H*-dibenz[*b*,*f*]azepine-5-carbamate (6)

GP-2, recrystallized from EtOH; yield 206 mg (70%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.51–7.27 (m, 8H, $H_{ar}$), 6.91 (s, 2H, $H_{ar}$), 3.92–3.70 (m, 2H, $CH_2$), 1.85–1.70 (m, 1H, CH), 0.76 (d, $J$ = 7 Hz, 6H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.3, 140.1, 134.4, 130.8, 130.2, 129.4, 129.1, 128.8, 127.4, 71.9, 28.0, 19.0; LC–MS ($m/z$): 294 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 4.10. *N*-Benzyl-5*H*-dibenz[*b*,*f*]azepine-5-carbamate (7)

GP-2, recrystallized from diethyl ether–petroleum ether; yield 187 mg (57%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.54–7.17 (m, 11H, $H_{ar}$), 7.18–7.06 (m, 2H, $H_{ar}$), 6.89 (d, $J$ = 1 Hz, 2H, $H_{ar}$), 5.10 (d, $J$ = 74 Hz, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.0, 140.2, 136.7, 134.4, 130.2, 129.4, 129.0, 128.7, 128.4, 127.8, 127.6, 127.3, 67.3; LC–MS ($m/z$): 328 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5. 5*H*-Dibenz[*b*,*f*]azepin-5-yl-4-morpholinyl-methanone (12)

GP-3, recrystallization from diethyl ether–petroleum ether; yield 231 mg (75%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.49 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.28–7.35 (m, 2H, $H_{ar}$), 7.22–7.12 (m, 4H, $H_{ar}$), 6.86 (s, 2H, $H_{ar}$), 3.33 (dd, $J$ = 5 Hz, $J$ = 5 Hz, 4H, $CH_2$), 3.03 (dd, $J$ = 5 Hz, $J$ = 5 Hz, 4H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 159.3, 142.6, 134.2, 131.4, 129.2, 129.0, 127.7, 126.6, 66.4, 46.5; LC–MS ($m/z$): 307 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5.1. *N*-[3-(Dimethylamino)propyl]-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (13)

GP-3, recrystallization from EtOAc; yield 202 mg (63%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.48–7.37 (m, 4H, $H_{ar}$), 7.37–7.27 (m, 4H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 6.03 (br s, 1H, NH), 3.28–3.21 (m, 2H, $CH_2$), 2.20 (t, $J$ = 6 Hz, 2H, $CH_2$), 1.90 (s, 6H, $CH_3$), 1.54–1.46 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.8, 140.7, 135.5, 130.6, 129.5, 129.4, 127.4, 59.2, 45.3, 41.2, 26.0; LC–MS ($m/z$): 322 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5.2. *N*-(*iso*-Propyl)-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (14)

GP-3, recrystallization from EtOAc; yield 187 mg (67%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.49–7.28 (m, 8H, $H_{ar}$), 6.91 (s, 2H, $H_{ar}$), 4.03 (d, $J$ = 7 Hz, 1H, NH), 3.97–3.83 (m, 1H, CH), 1.01 (d, $J$ = 7 Hz, 6H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.8, 140.4, 135.4, 130.6, 129.7, 129.4, 129.3, 127.6, 42.6, 23.3; LC–MS ($m/z$): 279 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5.3. *N*-(*p*-Methoxyphenylethyl)-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (15)

GP-3, recrystallization from EtOAc–EtOH; yield 305 mg (82%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.41–7.28 (m, 8H, $H_{ar}$), 6.98–6.94 (m, 2H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 6.80–6.75 (m, 2H, $H_{ar}$), 4.29 (t, $J$ = 6 Hz, 1H, NH), 3.79 (s, 3H, $OCH_3$), 3.33 (dd, $J$ = 13 Hz, $J$ = 7 Hz, 2H, $CH_2$), 2.65 (t, $J$ = 7 Hz, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 158.2, 156.3, 140.1, 135.4, 131.4, 130.6, 129.8, 129.7, 129.5, 129.3, 127.7, 114.0, 55.4, 42.1, 35.2; LC–MS ($m/z$): 371 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5.4. *N*-Cyclohexyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (16)

GP-3, recrystallization from EtOAc–petroleum ether; yield 247 mg (78%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.47–7.29 (m, 8H, $H_{ar}$), 6.91 (s, 2H, $H_{ar}$), 4.12 (d, $J$ = 8 Hz, 1H, NH), 3.65–3.53 (m, 1H, CH), 1.87–1.76 (m, 2H, $CH_2$), 1.58–1.46 (m, 3H, $CH_2$), 1.34–1.21 (m, 2H, $CH_2$), 1.13–1.01 (m, 1H, $CH_2$), 1.00–0.88 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.7, 140.4, 135.4, 130.6, 129.7, 129.4, 129.4, 127.6, 49.3, 33.5, 25.7, 24.8; LC–MS ($m/z$): 319 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 5.5. *N*-Phenylethyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (17)

GP-3, recrystallization from diethyl ether-petroleum ether; yield 264 mg (78%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.41–7.16 (m, 11H, $H_{ar}$), 7.04 (d, $J$ = 7 Hz, 2H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 4.29 (t, $J$ = 5, 1H, NH), 3.37 (td, $J$ = 7 Hz, $J$ = 7 Hz, 2H, $H_{ar}$), 2.71 (t, $J$ = 7 Hz, 2H, $H_{ar}$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.3, 140.1, 139.4, 135.4, 130.6, 129.7, 129.5, 129.3, 129.0, 128.6, 127.7, 126.4, 41.9, 36.2; LC–MS ($m/z$): 341 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6. 5*H*-Dibenz[*b*,*f*]azepin-5-yl-1-piperidinyl-methanone (18)

GP-3, recrystallzation from EtOAc; yield 285 mg (94%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.58 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.40–7.32(m, 2H, $H_{ar}$), 7.28–7.16 (m, 4H, $H_{ar}$), 6.93 (s, 2H, $H_{ar}$), 3.10–3.06 (m, 4H, $CH_2$), 1.46–1.34 (m, 2H, $CH_2$), 1.29–1.14 (m, 4H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 159.7, 143.4, 134.3), 131.5, 129.0, 128.9, 127.7, 126.2, 47.3, 25.3, 24.7; LC–MS ($m/z$): 305 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6.1. *N*,*N*-Dimethyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (20)

GP-3, recrystallization from EtOAc; yield 231 mg (88%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.56 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.40–7.33 (m, 2H, $H_{ar}$), 7.28–7.15 (m, 4H, $H_{ar}$), 6.94 (s, 2H, $H_{ar}$), 2.58 (s, 6H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 160.1, 143.2, 134.4, 131.5, 129.1, 128.9, 127.6, 126.2, 38.4; LC–MS ($m/z$): 265 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6.2. *N*-*n*-Butyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (21)

GP-3, recrystallization from EtOAc; yield 201 mg (69%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.49–7.29 (m, 8H, $H_{ar}$), 6.91 (s, 2H, $H_{ar}$), 4.25 (br s, 1H, NH), 3.18–3.06 (m, 2H, $CH_2$), 1.40–1.31 (m, 2H, $CH_2$), 1.28–1.15 (m, 2H, $CH_2$), 0.85 (t, $J$ = 7 Hz, 3H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.4, 140.3, 135.5, 130.6, 129.7, 129.5, 129.4, 127.7, 40.5, 32.3, 20.0, 13.9; LC–MS ($m/z$): 293 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6.3. *N*,*N*-Dipropyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (22)

GP-3, recrystallization from hexane; yield 236 mg (74%), colorless solid; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.56 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.37–7.30 (m, 2H, $H_{ar}$), 7.23 (dd, $J$ = 8 Hz, $J$ = 2 Hz, 2H, $H_{ar}$), 7.20–7.14 (m, 2H, $H_{ar}$), 6.92 (s, 2H, $H_{ar}$), 3.01–2.73 (m, 4H, $CH_2$), 1.28–1.13 (m, 4H, $CH_2$), 0.69 (t, $J$ = 7 Hz, 6H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 160.2, 143.4, 134.5, 131.5, 129.2, 128.9, 127.7, 126.2, 50.6, 20.9, 11.5; LC–MS ($m/z$): 321 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 99.4%.

### 6.4. *N*-[2-(Dimethylamino)ethyl]-*N*-methyl-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (23)

GP-3, recrystalli zation from diethyl ether–petroleum ether; yield 255 mg (79%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.56 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.37–7.31 (m, 2H, $H_{ar}$), 7.25–7.21 (m, 2H, $H_{ar}$), 7.20–7.15 (m, 2H, $H_{ar}$), 6.92 (s, 2H, $H_{ar}$), 3.26–3.17 (m, 2H, $CH_2$), 2.43 (s, 3H, $CH_3$), 2.21–2.09 (m, 8H, $CH_3$, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 159.7, 142.1, 134.3, 131.4, 129.4, 129.2, 127.4, 126.9, 54.4, 46.0, 43.5, 37.0; LC–MS ($m/z$): 322 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6.5. *N*-*tert*-Butyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (24)

GP-3, recrystallization from EtOAc; yield 211 mg (72%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.46–7.29 (s, 8H, $H_{ar}$), 6.92 (s, 2H, $H_{ar}$), 4.14 (s, 1H, NH), 1.21 (s, 9H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.6, 140.6, 135.4, 130.6, 129.8, 129.4, 129.4, 127.6, 51.0, 29.3; LC–MS ($m/z$): 293 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 99.7%.

### 6.6. *N*-(1-Methylpiperidin-4-yl)-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (25)

GP-3, recrystalli zation from EtOAc; yield 206 mg (62%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.48–7.29 (m, 8H, $H_{ar}$), 6.91 (s, 2H, $H_{ar}$), 4.10 (d, $J$ = 8 Hz, 1H, NH), 3.66–3.53 (m, 1H, CH), 2.56 (br s, 2H, $CH_2$), 2.19 (s, 3H, $CH_3$), 2.03 (t, $J$ = 9 Hz, 2H, $CH_2$), 1.89–1.78 (m, 2H, $CH_2$), 1.31–1.16 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.8, 140.2, 135.4, 130.6, 129.8, 129.5, 129.3, 127.8, 54.4, 47.1, 46.3, 32.6; LC–MS ($m/z$): 334 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 6.7. *N*,*N*-Dihydroxyethyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (26)

GP-3, recrystallization from diethyl ether–petroleum ether; yield 266 mg (82%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.53 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.40–7.35 (m, 2H, $H_{ar}$), 7.28–7.20 (m, 4H, $H_{ar}$), 6.94 (s, 2H, $H_{ar}$), 3.37 (dd, $J$ = 9, $J$ = 5 Hz, 4H, $CH_2$), 3.30 (t, $J$ = 5 Hz, 2H, OH), 3.20 (t, $J$ = 5 Hz, 4H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 161.7, 142.3, 134.3, 131.4, 129.5, 129.1, 127.6, 126.9, 61.1, 52.5; LC–MS ($m/z$): 325 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 7. 5*H*-Dibenz[*b*,*f*]azepin-5-yl-1-(4-hydroxy)piperidinyl-methanone (27)

GP-3, recrystallization from diethyl ether–petroleum ether; yield 244 mg (76%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.55 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.40–7.34 (m, 2H, $H_{ar}$), 7.27–7.18 (m, 4H, $H_{ar}$), 6.93 (s, 2H, $H_{ar}$), 3.69–3.59 (m, 1H, CH), 3.54–3.42 (m, 2H, $CH_2$), 2.81–2.70 (m, 2H, $CH_2$), 1.62–1.53 (m, 2H, $CH_2$), , 1.46 (d, $J$ = 4 Hz, 1H, OH), 1.22–1.09 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 159.4, 143.0, 134.2, 131.4, 129.1, 129.0, 127.7, 126.4, 67.9, 43.7, 33.8; LC–MS ($m/z$): 321 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 7.1. *N*-(4-Hydroxy-cyclohexyl)-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (28)

GP-3, recrystallization from EtOAc; yield 235 mg (70%), colorless prisms; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.50–7.27 (m, 8H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 4.03 (d, $J$ = 8 Hz, 1H, NH), 3.67–3.35 (m, 2H, 2CH), 1.97–1.78 (m, 4H, $CH_2$), 1.45 (d, $J$ = 4 Hz, 1H, OH), 1.37–1.22 (m, 2H, $CH_2$), 1.11–0.87 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.8, 140.2, 135.4, 130.6, 129.7, 129.5, 129.2, 127.7, 70.0, 48.9, 34.1, 31.2; LC–MS ($m/z$): 335 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 7.2. *N*-(4-Amino)cyclohexyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (29)

GP-3, recrystallization from MeOH-$H_2O$; yield 156 mg (54%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.48–7.27 (m, 8H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 4.02 (d, $J$ = 8 Hz, 1H, NH), 3.60–3.46 (m, 1H, CH), 2.56–2.48 (m, 1H, CH), 1.95–1.84 (m, 2H, $CH_2$), 1.83–1.69 (m, 2H, $CH_2$), 1.48 (br s, 2H, $NH_2$), 1.20–1.05 (m, 2H, $CH_2$), 1.03–0.87 (m, 2H, $CH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 155.8, 140.3, 135.4, 130.6, 129.7, 129.4, 129.3, 127.7, 50.0, 49.3, 35.5, 32.3; LC–MS ($m/z$): 291 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 7.3. *N*-(Pyridin-4-yl)-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (31)

GP-4, yield 131 mg (42%), colorless needles; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.35 (d, $J$ = 6 Hz, 2H, $H_{ar}$), 7.59–7.38 (m, 8H, $H_{ar}$), 7.20 (dd, $J$ = 5 Hz, $J$ = 2 Hz, 2H, $H_{ar}$), 6.98 (s, 2H, $H_{ar}$), 6.40 (s, 1H, NH); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 152.8, 150.5, 145.6, 138.9, 135.2, 130.6, 130.1, 129.9, 128.7, 128.5, 113.0. LC–MS ($m/z$): 314 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 7.4. 3-Methyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (43)

GP-6, recrystallization from EtOAc; yield 202 mg (81%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.50–7.23 (m, 6H, $H_{ar}$), 7.14 (dd, $J$ = 8, $J$ = 1 Hz, 1H, $H_{ar}$), 6.95–6.86 (m, 2H, $H_{ar}$), 4.55 (s, 2H, $NH_2$), 2.40 (s, 3H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 157.2, 140.1, 140.0, 135.3, 132.3, 130.5, 129.7, 129.5, 129.4, 129.2, 128.9, 128.8, 127.8, 21.2; LC–MS ($m/z$): 251 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 98.74%.

### 7.5. 3-Fluoro-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (44)

GP-6, recrystallization from EtOAc–EtOH; yield 178 mg (70%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.49–7.41 (m, 2H, $H_{ar}$), 7.39–7.29 (m, 3H, $H_{ar}$), 7.24–7.16 (m, 1H, $H_{ar}$), 7.08–7.02 (m, 1H, $H_{ar}$), 6.90 (s, 2H, $H_{ar}$), 4.65 (s, 2H, $NH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 163.4 (d, $J$ = 250 Hz), 156.9, 141.4 (d, $J$ = 10 Hz), 139.4, 135.3, 131.3, 130.7 (d, $J$ = 9 Hz), 130.0, 129.8 (d, $J$ = 6 Hz), 129.7, 128.7, 128.3, 116.2 (d, $J$ = 22 Hz), 115.4 (d, $J$ = 22 Hz); LC–MS ($m/z$): 255 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 99.68%.

### 7.6. 3-Trifluoromethyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (45)

GP-6, recrystallization from diethyl ether; yield 215 mg (71%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.77–7.72 (m, 1H, $H_{ar}$), 7.58–7.53 (m, 1H, $H_{ar}$), 7.53–7.44 (m, 3H, $H_{ar}$), 7.43–7.35 (m, 2H, $H_{ar}$), 7.03 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 6.97 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 4.61 (s, 2H, $NH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.7, 140.4, 139.9, 138.4, 134.9, 132.2, 131.5 (q, $J$ = 33 Hz), 130.3, 130.1, 130.1, 129.8, 128.7, 128.5, 126.5, 124.5 (q, $J$ = 3 Hz), 123.9 (q, $J$ = 272 Hz); LC–MS ($m/z$): 305 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 99.54%.

### 7.7. 3-Methoxy-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (46)

GP-6, recrystallization from EtOAc; yield 210 mg (79%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.50–7.18 (m, 5H, $H_{ar}$), 6.92–6.74 (m, 3H, $H_{ar}$), 4.57 (s, 2H, $NH_2$), 3.81 (s, 1H, OMe); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 161.1, 157.2, 141.2, 139.5, 135.5, 130.6, 130.3, 129.6, 129.3, 128.8, 128.4, 128.0, 114.7, 113.4, 55.7; LC–MS ($m/z$): 267 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 8. 5*H*-Benzo[*b*]naphtho[4,3-*f*]azepin-5-carboxamide (47)

GP-6, recrystallization from EtOAc; yield 220 mg (77%), colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 8.18 (d, $J$ = 8 Hz, 1H, $H_{ar}$), 7.91 (d, $J$ = 9 Hz, 1H, $H_{ar}$), 7.88–7.83 (m, 1H, $H_{ar}$), 7.70 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 7.60–7.49 (m, 4H, $H_{ar}$), 7.48–7.41 (m, 2H, $H_{ar}$), 7.37–7.32 (m, 1H, $H_{ar}$), 7.30–7.20 (m, 1H, $H_{ar}$), 4.59 (s, 2H, $NH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 157.0, 140.9, 135.4, 132.8, 131.6, 130.4, 129.8, 129.4, 128.7, 128.4, 127.9, 127.1, 126.6, 126.4, 124.3; LC–MS ($m/z$): 287 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 97.22%.

### 8.1. 3-Dimethylamino-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (48)

GP-6, recrystallization from diethyl ether; yield 190 mg (68%), pale yellow powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.38–7.32 (m, 3H, $H_{ar}$), 7.31–7.26 (m, 1H, $H_{ar}$), 7.24–7.19 (m, 1H), 6.85–6.78 (m, 1H), 6.75–6.63 (m, 3H, $H_{ar}$), 5.42 (s, 2H, $NH_2$), 3.28 (s, 6H, $H_{ar}$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 156.4, 151.6, 141.3, 139.7, 135.5, 130.2, 129.9, 129.4, 128.6, 128.3, 126.8, 126.1, 122.8, 111.6, 111.4, 39.9; LC–MS ($m/z$): 280 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 8.2. 10-(Methylamino)-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (58)

GP-7, yield 52%, colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.62 (d, $J$ = 8 Hz, 1H, $H_{ar}$), 7.52–7.43 (m, 2H, Har), 7.40–7.15 (m, 5H, $H_{ar}$), 5.72 (s, 1H, CH), 4.57 (s, 2H, $NH_2$), 3.89 (s, 1H, NH), 2.93 (s, 3H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 157.1, 147.0, 141.1, 138.0, 137.4, 134.7, 130.3, 129.2, 129.0, 128.1, 128.0, 127.4, 126.8, 125.9, 97.1, 31.1; LC–MS ($m/z$): 266 $[M+H]^+$; Purity by HPLC-UV (220–400 nm)-ESI-MS: 95.94%.

### 8.3. 10-Amino-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide (59)

GP-7, yield 46%, colorless powder; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.73 (d, $J$ = 8 Hz, 1H, $H_{ar}$), 7.52–7.44 (m, 2H, $H_{ar}$), 7.41–7.32 (m, 2H, $H_{ar}$), 7.25–7.14 (m, 3H, $H_{ar}$), 6.13 (s, 1H, CH), 4.57 (s, 2H, $NH_2$), 3.77 (s, 2H, $NH_2$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 157.1, 140.6, 138.2, 136.5, 134.1, 133.9, 130.6, 129.2, 128.6, 128.1, 128.0, 127.6, 126.8, 126.7, 103.4; LC–MS ($m/z$): 252 $[M+H]^+$; Purity by HPLC-UV (220–400 nm)-ESI-MS: 96.17%.

### 8.4. 4-(*N*,*N*-Diisopropyl)-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (32)

LDA (2 M, 3 mL) was added dropwsie to the solution of iminostilbene (580 mg, 3.00 mmol) and diisopropylcarbamoyl chloride (982 mg, 6.00 mmol) in dry THF (10 mL) at 0 °C. The reaction mixture was stirred under reflux for 0.5 h. After cooled down to room temperature, water (100 mL) was added. The mixture was extracted with dichloromethane (200 mL). The organic phase was separated, washed with brine (200 mL), and dried with $MgSO_4$. After evaporation of the solvent in vacuo, the residue was submitted to column chromatography ($SiO_2$, EtOAc–petroleum ether, 1:10) to give the product as orange prisms (490 mg, 51%). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.06–6.99 (m, 1H, $CH_{ar}$), 6.94–6.77 (m, 5H, $CH_{ar}$), 6.47–6.34 (m, 3H, $CH_{ar}$), 6.14 (s, 1H, NH), 4.06–3.47 (br d, 2H, CH), 1.85–0.80 (br d, 12 H, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 169.6, 149.0, 145.0, 133.0, 132.6, 132.0, 130.6, 130.5, 130.4, 129.7, 128.9, 126.1, 123.2, 122.3, 120.1, 51.2, 46.3, 20.9; LC–MS ($m/z$): 321 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 8.5. 4-(*N*,*N*-Diisopropylcarbamoyl)-*N*,*N*-diethyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (33)

the solution of triphosgene (445 mg, 1.5 mmol) in DCM (5 mL) was added to the solution of 4-(*N*,*N*-Diisopropyl)-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (**32**, 320 mg, 1.00 mmol) and DIEA (0.4 mL) in DCM (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h (TLC control). Diethylamine (1 mL) was added at 0 °C. It was stirred at room temperature for another 2 h. DCM (150 mL) was added to the reaction mixture. It was washed with HCl (1%, 200 mL), $NaHCO_3$ (5%, 200 mL), and brine (200 mL). The organic phase was dried with $MgSO_4$. After evaporation of the solvent in vacuo, the product was purified with column chromatography ($SiO_2$, EtOAc–petroleum ether, 1:5) as colorless prisms (320 mg, 76%). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.80 (dd, $J$ = 8, $J$ = 1 Hz, 1H), 7.44–7.38 (m, 1H, $H_{ar}$), 7.37–7.32 (m, 1H, $H_{ar}$), 7.32–7.28 (m, 1H, $H_{ar}$), 7.25–7.19 (m, 3H, $H_{ar}$), 7.01 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 6.92 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 4.02–3.90 (m, 1H, CH), 3.58–3.50 (m, 1H, CH), 3.19–3.08 (m, 2H, $CH_2$), 2.91–2.83 (m, 2H, $CH_2$), 1.68 (d, $J$ = 7 Hz, 3H, $CH_3$), 1.60 (d, $J$ = 7 Hz, 3H, $CH_3$), 1.13 (d, $J$ = 5 Hz, 3H, $CH_3$), 1.12 (d, $J$ = 5 Hz, 3H, $CH_3$), 0.64

(t, $J$ = 7 Hz, 6H, $CH_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 169.2, 159.5, 140.9, 138.8, 137.6, 136.8, 135.8, 131.4, 130.9, 129.6, 129.1, 128.9, 128.3, 126.8, 126.3, 125.7, 51.0, 45.7, 43.0, 21.0, 20.9, 20.9, 20.6, 12.9; LC–MS ($m/z$): 420 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 8.6. *N*,*N*-Diisopropyl-5*H*-dibenz[*b*,*f*]azepine-5-carboxamide (34)

The reaction mixture of dibenz[*b*,*f*]azepine (193 mg, 1.00 mmol), diisopropyl carbamoyl chloride (326 mg, 2.00 mmol), and DIEA (0.33 mL, 2.00 mmol) in toluene (15 mL) was stirred under Ar atmosphere at 140 °C for 24 h. After cooled down to room temperature, the volatile components were evaporated in vacuo. The product was purified with the column chromatography ($SiO_2$, EtOAc–petroleum ether, 1:30). The analytically pure sample was obtained by recrystallization from diethyl ether–petroleum ether as colorless prisms (176 mg, 55%). $^1H$ NMR (500 MHz, $CDCl_3$): $\delta$ 7.63 (dd, $J$ = 8, $J$ = 1 Hz, 2H, $H_{ar}$), 7.37–7.32 (m, 2H, $H_{ar}$), 7.25–7.15 (m, 4H, $H_{ar}$), 6.93 (s, 2H, $H_{ar}$), 3.59–3.45 (m, 2H, CH), 1.01 (d, $J$ = 6.5 Hz, 12H, $CH_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 159.0, 144.0, 134.8, 131.6, 129.2, 128.9, 127.5, 126.1, 48.1, 20.9; LC–MS ($m/z$): 321 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

### 8.7. 11-Diethylcarbamyl-benzo[*b*]pyrido[4,3-*f*]azepine (50)

Lithium bis(trimethylsilyl)amide (1 M, 2.00 mL, 2.00 mmol) was added dropwise to the solution of 11*H*-Benzo[*b*]pyrido[4,3-*f*]azepine (**42**, 194 mg, 1.00 mmol) and *N*,*N*-diethylcarbamoyl chloride (2.00 mmol, 272 mg) in dry THF (5 mL) at 0 °C. The reaction mixture was stirred under Ar atmosphere at room temperature for 2 h. Methanol (1 mL) was added to the reaction mixture. The volatile components were evaporated in vacuo. The residue was submitted to column chromatography ($SiO_2$, EtOAc–petroleum ether, 1:1). The analytically pure sample was obtained by purification with preparative reverse phase HPLC. Yield 70 mg (24%), pale yellow solid. $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 8.89 (s, 1H, $H_{ar}$), 8.39 (d, $J$ = 5 Hz, 1H, $H_{ar}$), 7.61–7.53 (m, 1H, $H_{ar}$), 7.47–7.36 (m, 1H, $H_{ar}$), 7.32–7.20 (m, 2H, $H_{ar}$), 7.13 (d, $J$ = 5 Hz, 1H, $H_{ar}$), 7.10 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 6.87 (d, $J$ = 12 Hz, 1H, $H_{ar}$), 3.06 (q, $J$ = 7 Hz, 4H, $CH_2$), 0.78 (t, $J$ = 7 Hz, 6H, $CH_3$); $^{13}C$ NMR (125 MHz, $CDCl_3$): $\delta$ 159.5, 149.4, 146.6, 144.0, 141.3, 139.1, 135.6, 133.7, 130.2, 129.7, 129.3, 127.7, 126.7, 122.3, 42.7, 12.5; LC–MS ($m/z$): 294 $[M+H]^+$. Purity by HPLC-UV (220–400 nm)-ESI-MS: 100%.

## 9. Biological assays

All biological assays were performed as previously described.[22]

## Acknowledgments

We thank Marion Schneider for LCMS analyses, Sabine Terhart-Krabbe and Annette Reiner for NMR spectra, Ralf Hausmann and Günter Schmalzing (University of Aachen, Germany) for the gift of some P2X receptor clones, and Florence Moureau and colleagues, UCB Pharma, for helpful suggestions. This study was supported by the BMBF (German Federal Ministry for Education and Research) within the BioPharma initiative 'Neuroallianz'.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.035.

## References and notes

1. Burnstock, G. *Front. Biosci.* **2011**, *3*, 896.
2. Burnstock, G. *BioEssays* **2012**, *34*, 218.
3. Coddou, C.; Yan, Z.; Obsil, T.; Huidobro-Toro, J. P.; Stojilkovic, S. S. *Pharmacol. Rev.* **2011**, *63*, 641.
4. Abbracchio, M. P.; Burnstock, G.; Boeynaems, J. M.; Barnard, E. A.; Boyer, J. L.; Kennedy, C.; Knight, G. E.; Fumagalli, M.; Gachet, C.; Jacobson, K. A.; Weisman, G. A. *Pharmacol. Rev.* **2006**, *58*, 281.
5. Kawate, T.; Michel, J. C.; Birdsong, W. B.; Gouaux, E. *Nature* **2009**, *460*, 592.
6. Hattori, M.; Gouaux, E. *Nature* **2012**, *485*, 207.
7. Nicke, A.; Bäumert, H. G.; Rettinger, J.; Eichele, A.; Lambrecht, G.; Mutschler, E.; Schmalzing, G. *EMBO J.* **1998**, *17*, 3016.
8. Inoue, K.; Tsuda, M. *Exp. Neurol.* **2012**, *234*, 293.
9. Beggs, S.; Salter, M. W. *Neurosci. Lett.* **2013**, *557*, 37.
10. Inoue, K.; Tsuda, M. *CNS Neurol. Disord.: Drug Targets* **2012**, *11*, 699.
11. Beggs, S.; Trang, T.; Salter, M. W. *Nat. Neurosci.* **2012**, *15*, 1068.
12. Ulmann, L.; Hatcher, J. P.; Hughes, J. P.; Chaumont, S.; Green, P. J.; Conquet, F.; Buell, G. N.; Reeve, A. J.; Chessell, I. P.; Rassendren, F. *J. Neurosci.* **2008**, *28*, 11263.
13. Chen, K.; Zhang, J.; Zhang, W.; Zhang, J.; Yang, J.; Li, K.; He, Y. *Int. J. Biochem. Cell Biol.* **2013**, *45*, 932.
14. Li, F.; Guo, N.; Ma, Y.; Ning, B.; Wang, Y.; Kou, L. *Inflammation* **2013**, in press, doi: http://dx.doi.org/10.1007/s10753-013-9723-y.
15. Lu, W. H.; Wang, C. Y.; Chen, P. S.; Wang, J. W.; Chuang, D. M.; Yang, C. S.; Tzeng, S. F. *J. Neurosci. Res.* **2013**, *91*, 694.
16. Ulmann, L.; Levavasseur, F.; Avignone, E.; Peyroutou, R.; Hirbec, H.; Audinat, E.; Rassendren, F. *Glia* **2013**, *61*, 1306.
17. Müller, C. E. *Purinergic Signal.* **2010**, *6*, 145.
18. Gum, R. J.; Wakefield, B.; Jarvis, M. F. *Purinergic Signal.* **2012**, *8*, S41.
19. Gunosewoyo, H.; Kassiou, M. *Expert Opin. Ther. Patents* **2010**, *20*, 625.
20. Nagata, K.; Imai, T.; Yamashita, T.; Tsuda, M.; Tozaki-Saitoh, H.; Inoue, K. *Mol. Pain* **2009**, *5*, 20.
21. Toulme, E.; Garcia, A.; Samways, D.; Egan, T. M.; Carson, M. J.; Khakh, B. S. *J. Gen. Physiol.* **2010**, *135*, 333.
22. Hernandez-Olmos, V.; Abdelrahman, A.; El-Tayeb, A.; Freudendahl, D.; Weinhausen, S.; Müller, C. E. *J. Med. Chem.* **2012**, *55*, 9576.
23. Ambrosio, A. F.; Soares-da-Silva, P.; Carvalho, C. M.; Carvalho, A. P. *Neurochem. Res.* **2002**, *27*, 121.
24. Dahlgren, T.; Hallberg, A.; Helitzer, R.; Martin, A. R. *J. Heterocycl. Chem.* **1983**, *20*, 341.
25. (a) Kricka, L. J.; Ledwith, A. *Chem. Rev.* **1974**, *74*, 101; (b) Tokmakov, G. P.; Grandberg, I. I. *Tetrahedron* **1995**, *51*, 2091; (c) Craig, P. N.; Lester, B. M.; Saggiomo, A. J.; Kaiser, C.; Zirkle, C. L. *J. Org. Chem.* **1961**, *26*, 135; (d) Monti, K. D.; Maciejewski, A. B. *Appl. Catal. A* **1995**, *121*, 139.
26. Singh, H.; Gupta, N.; Kumar, P.; Dubey, S. K.; Sharma, P. K. *Org. Process Res. Dev.* **2009**, *13*, 870.
27. Tsvelikhovsky, D.; Buchwald, S. L. *J. Am. Chem. Soc.* **2010**, *132*, 14048.
28. Eckardt, R.; Jaensch, H.-J. U.S. Patent 7015322, 2006.
29. Doyagüez, E. G. *Synlett* **2005**, 1636.
30. Rosowsky, A.; Fu, H.; Chan, D. C.; Queener, S. F. *J. Med. Chem.* **2004**, *47*, 2475.
31. Maggs, J. L.; Pirmohamed, M.; Kitteringham, N. R.; Park, B. K. *Drug Metab. Dispos.* **1997**, *25*, 275.
32. Kaczmarek-Hájek, K.; Lörinczi, E.; Hausmann, R.; Nicke, A. *Purinergic Signal.* **2012**, *8*, 375.
33. Gaddum, J. H.; Hameed, K. A.; Hathaway, D. E.; Stephens, F. F. *Q. J. Exp. Physiol.* **1955**, *40*, 49.
34. Vauquelin, G.; Van Liefde, I.; Birzbier, B. B.; Vanderheyden, P. M. *Fundam. Clin. Pharmacol.* **2002**, *16*, 263.
35. Landek, I. O.; Pesic, D.; Mercep, M.; Stanic, B.; Mesic, M. *J. Heterocycl. Chem.* **2011**, *48*, 856.
36. Crawford, L. A.; McNab, H.; Mount, A. R.; Wharton, S. I. *J. Org. Chem.* **2008**, *73*, 6642.
37. Shieh, W.-C.; Dell, S.; Bach, A.; Repic, O.; Blacklock, T. J. *J. Org. Chem.* **1954**, *2003*, 68.
38. Schindler, W. U.S. Patent 2948718 19600809, 1960.
39. Kawashima, K.; Ishiguro, T. *Ger. Offen.* DE 2246842 A1 19730405, 1973.
40. Kulkarni, G. H.; Naik, R. H.; Tandel, S. K.; Rajappa, S. *Tetrahedron* **1991**, *47*, 1249.
41. Balaure, P. C.; Costea, I.; Iordache, F.; Draghici, C.; Enache, C. *Rev. Roum. Chim.* **2009**, *54*, 935.
42. Aziz-Ur-Rahman; Fareedullah, Md.; Rizwan, S. H.; Abdul Mateen, S. *Int. J. Pharm. Technol.* **2012**, *4*, 4423.
43. Lucini, V.; Pannacci, M.; Scaglione, F.; Fraschini, F.; Rivara, S.; Mor, M.; Bordi, F.; Plazzi, P. V.; Spadoni, G.; Bedini, A.; Piersanti, G.; Diamantini, G.; Tarzia, G. *J. Med. Chem.* **2004**, *47*, 4202.
44. Della Ca', N.; Maestri, G.; Malacria, M.; Derat, E.; Catellani, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 12257.
45. Elliott, E.-C.; Bowkett, E. R.; Maggs, J. L.; Bacsa, J.; Park, B. K.; Regan, S. L.; O'Neill, P. M.; Stachulski, A. V. *Org. Lett.* **2011**, *13*, 5592.
46. Elliott, E.-C.; Regan, S. L.; Maggs, J. L.; Bowkett, E. R.; Parry, L. J.; Williams, D. P.; Park, B. K.; Stachulski, A. V. *J. Med. Chem.* **2012**, *55*, 9773.
47. Chang, V. H. T. *J. Heterocycl. Chem.* **1983**, *20*, 237.
48. Learmonth, David Alexander, U.S. Patent 7241886, 2007.