Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry. This journal is © The Royal Society of Chemistry 2014

# **Supporting Information**

# Developing Piperine towards TRPV1 and GABA<sub>A</sub> Receptor Ligands -Synthesis of Piperine Analogs *via* Heck-Coupling of Conjugated Dienes

#### Laurin Wimmer, David Schönbauer, Peter Pakfeifer, Angela Schöffmann, Sophia Khom, Steffen Hering and Marko D. Mihovilovic

Unless otherwise noted, chemicals were purchased from commercial suppliers and used without further purification. Microwave reactions were performed on a Biotage Initiator Sixty microwave unit (Biotage AB, Uppsala, Sweden). Flash column chromatography was performed on silica gel 60 from Merck (40–63mm), whereas most separations were performed by using a Büchi SepacoreTM MPLC system with a 9g column (Buchi Labortechnik AG, Flawil, Switzerland). Evaporation of solvents was carried out either on standard rotary evaporators or on a Christ RVC 2-25plus centrifugal evaporator with attached cooling system (Christ 2-4 LOplus). Aluminum-backed silica gel was used for TLC. Melting points were determined by using a Kofler-type Leica Galen III micro hot stage microscope (Aigner-Unilab Laborfachhandel GmbH, Vienna, Austria) and are uncorrected. For compounds unknown in the literature high-resolution MS was performed by LC-IT-TOF-MS (Shimadzu) in positive or negative ion detection mode with the recording of MS and MS/MS spectra. NMR-spectra were recorded on a Bruker AC 200 (200 MHz) or a Bruker Avance 400 (400MHz) spectrometer (Bruker GmbH, Vienna, Austria) and chemical shifts are reported in ppm. For assignment of 13C multiplicities standard 13C, DEPT or APT spectra were recorded. GC analyses were performed on a Thermo Finnigan Focus GC with FID detector and a BGB5 column (30m x 0.32mm ID, 1.0µm) using a standard temperature program (2min at 100°C, 18°C/min unitl 280°C, 10min at 280°C). GC-MS runs were performed on a Thermo Finnigan Focus GC/DSQ II with a standard capillary column BGB 5 (ID=30m, 0.32 mm; Fisher Scientific GmbH, Vienna, Austria) using standardized temperature programs: "short method" (2min at 100°C, 18°C/min until 280°C, 3min at 280°C) and "long method" (2min at 100°C, 18°C/min until 280°C, 10min at 280°C).

#### Abbreviations

MeCN	acetonitrile
brine	saturated aqueous NaCl-solution
dba	dibenzylideneacetone
DCM	methylenchloride
DMF	dimethylformamide
EDCI·HCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
equiv.	equivalent
LP	light petroleum
M.p.	melting point
MPLC	medium-preassure liquid chromatography
rt	room temperature
TLC	thin layer chromatography
HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

## Analytical protocol for reaction screening

#### Calibration

GC was calibrated with samples of 4-bromotoluene, (*E*)-penta-2,4-dienoic dibutylamide and (*2E,4E*)-*N*,*N*-dibutyl-5-(p-tolyl)penta-2,4-dienamide with concentrations of 0.3, 0.75, 1.5, 3.0, 4.2 and 6.0mM in acetonitrile. Methylbenzoate (3.0mM) was used as internal standard. All compounds were found to have a linear response over the whole concentration range.

#### Sample preparation

Reaction mixtures were filtered (through cotton and Celite<sup>®</sup>), the vials and filters were washed thoroughly with small quantities of MeCN. 1ml of a 150mM solution of methylbenzoate in MeCN was added to the filtrate. The filtrate was diluted with MeCN to give a total volume of 10.0ml. GC-samples were taken from this solution and further diluted in a ratio of approximately 1:4, giving a final concentration of ~1mg/ml. All samples were filtered through PALL Acrodisc<sup>®</sup> CR 13mm syringe filters with 0.2µm PTFE membrane prior to GC-analysis.

## Screening results for Pd(dba)<sub>2</sub> as palladium-source

The reactions summarized in Table 1 were performed according to the general coupling procedure using 2mol% of Pd(dba)<sub>2</sub> and 4mol% of monodentate or 2mol% of bidentate ligands.

Entry	Solvent	Base	Pd-source	Ligand	T/°C	Time	GC-Yield
1	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	(o-tolyl) <sub>3</sub> P	140	1h	80%
2	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	(2-furyl) <sub>3</sub> P	140	1h	7%
3	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	(p-ClPh) <sub>3</sub> P	140	1h	59%
4	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	(1-naphtyl) <sub>3</sub> P	140	1h	66%
5	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	dppf	140	1h	75%
6	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	Cy <sub>3</sub> P	140	1h	44%
7	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	dppp	140	1h	42%
8	DMF	K <sub>2</sub> CO <sub>3</sub>	Pd(dba) <sub>2</sub>	JohnPhos	140	1h	77%

#### Table 1: Screening results Pd(dba)<sub>2</sub>

# Compound 1, (E)-penta-2,4-dienoic acid

Compound **1** was prepared according to a procedure<sup>1</sup> published by Brimble et al. A solution of malonic acid (1 equiv., 19.99 g, 192 mmol) in pyridine (1.9 equiv, 28.91 g, 365 mmol) was heated to reflux. Acrolein (1.35 equiv., 14.53 g, 259 mmol) was added dropwise over a period of about 30 min and the reaction was heated to reflux for another 30 min. The reaction mixture was cooled to rt, poured into 130 ml of ice and adjusted to pH = 1 with  $H_2SO_4$ . The product precipitated as yellow solid and the suspension was extracted with DCM (3 x 160 ml). The combined organic layers were washed with brine. Evaporation of the solvent gave the crude product (crude yield: 74 %), which contained significant amounts of polymerized product as judged by NMR. The pure product was obtained after recrystallization from ligroin.

Yield:	42% (80.1mmol, 7.86 g) (lit.: 72 %) <sup>1</sup>
Appearance:	yellowish crystals
TLC:	0.45 (CHCl <sub>3</sub> /MeOH 9.1)
M.p.:	69 - 71°C (lit.: 68 to 70°C) <sup>2</sup>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>): δ = 5.57 (d, J = 10.0Hz, 1H), 5.67 (d, J = 16.9Hz, 1H), 5.92 (d, J = 15.4Hz, 1H), 6.49 (dt, J = 16.9Hz, J = 10.4Hz, 1H), 7.36 (dd, J<sub>1</sub> = 15.4Hz, J<sub>2</sub> = 11.0Hz, 1H), 11.80 (s, 1H) <sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>): δ = 121.3 (d), 126.8 (t) 134.5 (d) 147.1 (d) 172.6 (s)

# Compound 2, (E)-penta-2,4-dienoic dibutylamide

#### Synthesis with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI)

Dibutylamine (1.09 equiv , 705 mg, 5.46 mmol, freshly distilled over CaH<sub>2</sub>) and 1-ethyl-3-(3dimethylaminopropyl)carbodiimid hydrochloride (1.1 equiv, 1048 mg, 5.47 mmol) were added successively to a solution of (*E*)-penta-2,4-dienoic acid (1 equiv, 488 mg, 5mmol) in anhydrous DCM (23ml). The reaction mixture was stirred at rt for 1.5 h, when TLC (MeOH/CHCl<sub>3</sub> 9:1) indicated full consumption of the starting material. The reaction was diluted with 50ml of DCM and washed with 0.5 M HCl (2x50 ml), NaHCO<sub>3</sub> sat. (25 ml) and brine (25 ml), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to give the pure product.

Yield: 62 % (3.08 mmol, 646.1 mg)

#### Synthesis with oxalyl chloride

(*E*)-Penta-2,4-dienoic acid (1.00 equiv., 11.08 g, 113 mmol) was dissolved in 51 ml dry DCM under argon. A few drops of DMF were added, followed by the dropwise addition of oxalyl chloride (0.95 equiv ., 13.68 g, 108 mmol) via syringe over a period of 15 min. Stirring continued for 1.5 h at rt to ensure full conversion to the acid chloride.

A solution of dibutylamine (1.50 equiv ., 21.95g, 170mmol, freshly distilled over  $CaH_2$ ) and triethylamine (1.00 equiv ., 11.42g, 113 mmol) in 100 ml of anhydrous DCM was cooled to -70 °C under argon atmosphere. The solution of the acid chloride was added dropwise while maintaining the temperature at -70 to -60°C. After stirring at this temperature for another 20min the reaction mixture was allowed to warm to rt.

The reaction mixture was washed with 3x 150 ml 0.5M HCl, satd. NaHCO<sub>3</sub> (2x150 ml) and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated to give the pure product. From the aqueous phase 3.3 g of (*E*)-penta-2,4-dienoic acid could be recovered.

Yield: 33% (24.7 mmol, 5.18 g)Appearance:pale yellow oilTLC:0.87 (CHCl<sub>3</sub>/MeOH 9:1)

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.80-1.00 (m, 6H), 1.21-1.42 (m, 4H), 1.43-1.65 (m, 4H), 3.17-3.46 (m, 4H), 5.38 (d, J = 10.1Hz, 1H), 5.53 (d, J = 16.8Hz, 1H), 6.29 (d, J = 14.9Hz, 1H), 6.46 (dt, J<sub>1</sub> = 16.9Hz, J<sub>2</sub> = 10.5Hz, 1H), 7.27 (dd, J<sub>1</sub> = 14.9Hz, J<sub>2</sub> = 10.9Hz, 1H).

 $^{13}\text{C-NMR}$  (50 MHz, CDCl<sub>3</sub>): 13.8 (q), 13.9 (q), 20.0 (t), 20.3 (t), 30.0 (t), 31.9 (t), 46.5 (t) 47.9 (t), 121.8 (d) 123.7 (t) 135.3 (d) 142.4 (d) 165.9 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 210.1852, m/z (measured) = 210.1852, difference = 0.0 ppm

GC - MS: short method,  $t_R = 7.79$  min, main fragments: 209 (10, M<sup>+</sup>), 166 (25), 128 (11), 86 (12), 81 (100)

#### **General Coupling Procedure**

An 8ml screw-cap vials was charged with  $K_2CO_3$  (159mg, 1.15mmol, 3equiv.) and arylhalide (0.383mmol, 1.0 equiv.). Then (*E*)-penta-2,4-dienoic dibutylamide (80.2mg, 0.383mmol, 1equiv.), dppp (3.14mg, 2mol%) and Pd(OAc)<sub>2</sub> (1.74mg, 2mol%) were added as a stock-solution in degassed DMF. DMF was added to adjust the concentration to 3%w/v with respect to the olefin (2.7ml DMF total volume). The solution was degassed by argon sparging for 5min and heated for 1 h at 140°C. GC-MS indicated full consumption of the halide. The reaction mixture was cooled to rt, filtered through Celite<sup>®</sup> and evaporated. The crude product was purified by column chromatography on silica.

#### Compound 4, (2E,4E)-N,N-dibutyl-5-phenylpenta-2,4-dienamide

Halide:	bromobenzene (0.39 mmol, 61.7 mg)
Yield:	73% (0.277mmol, 79.3 mg)
Appearance:	off-white solid
M.p.:	46-48 °C
TLC:	0.22 (PhMe/EtOAc 9:1)
MPLC:	PhMe/EtOAc 90:10, 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.79-1.12 (m, 6H), 1.18-1.46 (m, 4H), 1.46-1.75 (m, 4H), 3.17-3.57 (m, 4H), 6.40 (d, <sup>3</sup>J = 14.6Hz, 1H), 6.74-7.08 (m, 2H), 7.18-7.40 (m, 3H), 7.40-7.61 (m, 3H).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.86 (q), 13.92 (q), 20.1 (t), 20.3 (t), 30.1(t), 31.9(t), 46.6 (t), 47.9 (t), 121.1 (d), 126.9 (d), 127.0 (d), 128.5 (d), 128.7 (d), 136.5 (s), 138.6 (d), 142.3 (d), 166.0 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 286.2165, m/z (measured) = 286.2154, difference = -3.84 ppm

GC - MS: short method,  $t_R = 12.24$  min, main fragments 285 (10, M<sup>+</sup>), 242 (8), 157 (100), 128 (52), 115 (9)

# Compound 5, (2E,4E)-N,N-dibutyl-5-(4-methylphenyl)penta-2,4-dienamide

For the synthesis of **5** a slightly modified procedure was used. (*E*)-penta-2,4-dienoic dibutylamid (1.00 equiv., 25.84 mg, 0.123 mmol), bromotoluene (1.00 equiv., 21.0 mg, 0.123 mmol), Pd(OAc)<sub>2</sub> (2 mol %, 0.52 mg), dppp (2.0 mol %, 1.41 mg) and K<sub>2</sub>CO<sub>3</sub> (3.0 equiv., 51.1 mg, 0.37 mmol) were added in a vial and diluted in 1ml DMF, set under argon and heated at 140°C for 1h. GC-MS indicated full conversion of the starting material and the product was extracted with Diethylether. The organic phase was pre-dried with brine and dried with Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent the pure product was isolated by MPLC.

Yield:	83% (0.102 mmol, 30.6 mg)
Appearance:	colorless oil
TLC:	0.37 (LP/EtOAc 9:1)
MPLC:	toluene/EtOAc 10:1, 9g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.78-1.05 (m, 6H), 1.20-1.46 (m, 4H), 1.46-1.70 (m, 4H), 2.35 (s, 3H), 3.17-3.57 (m, 4H), 6.37 (d, J = 14.6Hz, 1H), 6.67-6.98 (m, 2H), 7.15 (d, J = 8.0Hz, 2H), 7.36 (d, J = 8.0Hz, 2H), 7.39 - 7.56 (m, 1H).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 21.3 (q), 30.1 (t), 31.9 (t), 46.6 (t), 47.9 (t), 120.5 (d), 126.1 (d), 126.8 (d), 129.4 (d), 133.7 (s), 138.6 (s), 138.7 (d), 142.5 (d), 165.1 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 300.2322, m/z (measured) = 300.2328, difference = 2.00 ppm

GC - MS: short method,  $t_R = 13.09$  min, main fragments 299 (10, M<sup>+</sup>), 256 (7), 171 (100), 128 (65), 115 (15).

#### Compound 6, (2E,4E)-N,N-dibutyl-5-(4-methoxyphenyl)penta-2,4-dienamide

Halide:	1-bromo-4-methoxybenzen (0.39 mmol, 72.8 mg)
Yield:	64% (0.24mmol, 76.8 mg)
Appearance:	colorless oil.
TLC:	0.15 (LP/EtOAc 5:1)
MPLC:	heptane/EtOAc 17:3; 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz,  $CDCl_3$ ):  $\delta$  = 0.77-1.03 (m, 6H), 1.17-1.44 (m, 4H), 1.45-1.71 (m, 4H), 3.18-3.51 (m, 4H), 3.80 (s, 3H), 6.33 (d, J = 14.6Hz, 1H), 6.71-6.94 (m, 4H), 7.29-7.64 (m, 3H).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.85 (q), 13.90 (q), 20.1 (t), 20.3 (t), 30.1 (t), 32.0 (t), 46.6 (t), 47.9 (t), 55.3 (q), 114.2 (d), 119.9 (d), 125.0 (d), 128.3 (d), 129.3 (s), 138.3 (d), 142.7 (d), 160.0 (s), 166.2 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 316.2271, m/z (measured) = 316.2273, difference = 0.63 ppm

GC - MS: long method,  $t_R$  = 13.61 min, main fragments 315 (13, M<sup>+</sup>), 187 (100), 144 (36), 128 (29), 115 (34).

#### Compound 7, (2E,4E)-N,N-dibutyl-5-(3-methoxyphenyl)penta-2,4-dienamide

Halide:	3-bromoanisole (0.38 mmol, 72.0 mg)
Yield:	84% (0.321 mmol, 101.2 mg)
Appearance:	colorless oil
TLC:	0.19 (PhMe/EtOAc 95:5) and 0.40 (LP/EtOAc 80:20)
MPLC:	PhMe/EtOAc 95:5, 9g SiO <sub>2</sub> or LP/EtOAc 9:1, 9g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.81-1.00 (m, 6H), 1.21-1.46 (m, 4H), 1.46-1.73 (m, 4H), 3.17-3.57 (m, 4H), 3.74 (s, 3H), 6.40 (d, J = 14.6Hz, 1H), 6.74-6.93 (m, 3H), 6.93-7.00 (m, 1H), 7.00-7.12 (m, 1H), 7.19-7.32 (m, 1H), 7.47 (dd, J<sub>1</sub> = 9.54Hz, J<sub>2</sub> = 14.5Hz, 1H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.85 (q), 13.91 (q), 20.1(t), 20.3 (t), 30.1(t), 31.9(t), 46.6 (t), 47.9(t), 55.2 (q), 112.0 (d), 114.4 (d), 119.6 (d), 121.2 (d), 129.7 (d), 137.9 (s), 138.5 (d), 142.2 (d), 159.8 (s), 166.0 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 316.2271, m/z (measured) = 316.2269, difference = -0.63 ppm

GC - MS: short method,  $t_R = 13.71$  min, main fragments 315 (10, M<sup>+</sup>), 272 (8), 187 (100), 115 (40), 57 (11)

#### Compound 8, (2E,4E)-N,N-dibutyl-5-(3,5-dimethoxyphenyl)penta-2,4-dienamide

Halide:	1-bromo-3,5-dimethoxybenzen (0.38 mmol, 83.2 mg)
Yield:	61% (0.23 mmol, 81.0 mg)
Appearance:	colorless oil
TLC:	0.13 (LP/EtOAc 5:1)
MPLC:	heptane/EtOAc 17:3; 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.78-1.01 (m, 6H), 1.19-1.43 (m, 4H), 1.43-1.70 (m, 4H), 3.16-3.47 (m, 4H), 3.77 (s, 6H), 6.37 (d, J = 14.7Hz, 1H), 6.37 (t, J = 2.2Hz, 1H), 6.57 (d, J = 2.3Hz, 2H), 6.73 (d, J = 15.5Hz, 2H), 6.87 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 10.1Hz, 1H), 7.42 (dd, J<sub>1</sub> = 14.7Hz, J<sub>2</sub> = 10.0Hz, 1H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.1 (t), 32.0 (t), 46.6 (t), 47.9 (t), 55.3 (q), 101.0 (d) 104.9 (d), 121.4 (d), 127.5 (d), 138.4 (s), 138.6 (d), 142.0 (d), 160.9 (s), 166.0 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 346.2377, m/z (measured) = 346.2360, difference = -4.91 ppm

GC - MS: long method,  $t_R$  = 15.28 min, main fragments 345 (8, M<sup>+</sup>), 217 (48), 189 (100), 175 (45), 174 (26).

#### Compound 9, (2E,4E)-N,N-dibutyl-5-(4-(tert-butyl)-phenyl)penta-2,4-dienamide

Halide:	1-bromo-4-tert-butylbenzene (0.39 mmol, 82.3 mg)
Yield:	64% (0.25 mmol <i>,</i> 84.2 mg)
Appearance:	off-white crystals
M.p.:	52-53°C
TLC:	0.31 (PhMe/EtOAc 9:1)
MPLC:	PhMe/EtOAc 95:5, 9g SiO₂

<sup>1</sup>H-NMR (200 MHz,  $CDCI_3$ ):  $\delta$  = 0.89-1.00 (m, 6H), 1.18-1.46 (m, 13H), 1.46-1.73 (m, 4H), 3.17-3.57 (m, 4H), 6.38 (d, J = 14.5Hz, 1H), 6.70-7.00 (m), 7.28-7.56(m).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>)  $\delta$  =13.86 (q), 13.93 (q), 20.1 (t), 20.3 (t), 30.1 (t), 31.2 (q), 31.9 (t), 34.7 (s), 46.6 (t), 47.9 (t), 120.5 (d), 125.7 (d), 126.3 (d), 126.7 (d), 133.7 (s), 138.6 (d), 142.6 (d), 151.9 (s), 166.1 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 342.2791, m/z (measured) = 342.2788, difference = -0.88 ppm

GC - MS: short method,  $t_R = 14.65$  min, main fragments 341 (17, M<sup>+</sup>), 213 (44), 157 (32), 85 (27), 57 (100).

## Compound 10, (2E,4E)-N,N-dibutyl-5-(4-chlorophenyl)penta-2,4-dienamide

Halide:	1-bromo-4-chlorobenzen (0.38 mmol, 73.3 mg)
Yield:	60% (0.23 mmol, 72.8 mg)
Appearance:	yellow oil
TLC:	0.26 (LP/EtOAc 5:1)
MPLC:	heptane/EtOAc 10:1, 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.75-0.99 (m, 6H), 1.17-1.40 (m, 4H), 1.40-1.65 (m, 4H), 3.19-3.43 (m, 4H), 6.35 (d, J = 14.5Hz, 1H), 6.71 (d, J = 15.3Hz, 1H), 6.84 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 9.7Hz, 1H), 7.16 - 7.49 (m)

<sup>13</sup>C-NMR (50 MHz, CDCI<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.2 (t), 32.0 (t), 46.7 (t), 48.1 (t), 121.6 (d), 127.6 (d), 128.1 (d), 128.9 (d), 134.2 (s), 135.0 (s), 137.1 (d), 141.9 (d) 165.5 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 320.1776, m/z (measured) = 320.1789, difference = 4.06 ppm

GC - MS: long method,  $t_R = 13.09$  min, main fragments 319 (16, M+), 276 (10), 191 (100), 152 (12), 128 (50).

#### Compound 11, (2E,4E)-N,N-dibutyl-5-(3-chlorophenyl)penta-2,4-dienamide

Halide:	1-bromo-3-chlorobenzene (0.38 mmol, 72.6 mg)
Yield:	68% (0.256 mmol, 81.9 mg)
Appearance:	pale green oil
TLC:	0.28 (PhMe/EtOAc 9:1)
MPLC:	PhMe/EtOAc 90:10, 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCI<sub>3</sub>):  $\delta$  = 0.78-1.06 (m, 6H), 1.22-1.47 (m, 4H), 1.47-1.71 (m, 4H), 3.21-3.51 (m, 4H), 6.44 (d, J = 14.6Hz, 1H), 6.76 (d, J = 15.5Hz, 1H), 6.92 (dd, J<sub>1</sub> = 10.4Hz, J<sub>2</sub> = 15.5Hz, 1H), 7.17-7.35 (m, 3H), 7.35-7.54 (m, 2H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.1 (t), 32.0 (t), 46.6 (t), 47.9 (t), 122.1 (d), 125.3 (d), 126.5 (d), 129.3 (d), 128.4 (d), 129.9 (d), 134.7 (s), 136.8 (d), 138.3 (s), 141.6 (d), 165.8 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 320.1776, m/z (measured) = 320.1780, difference = 1.25 ppm

GC - MS: short method, t<sub>R</sub> = 13.31 min, main fragments 319 (10, M<sup>+</sup>), 276 (9), 207 (30), 191 (100), 128 (89).

#### Compound 12, (2E,4E)-N,N-dibutyl-5-(4-(trifluoromethyl)phenyl)penta-2,4-dienamide

Halide:	1-bromo-4-(trifluoromethyl)benzene (0.385 mmol, 86.8 mg)
Yield:	68% (0.261 mmol, 92.1 mg)
Appearance:	pale green solid
M.p.:	67 °C
TLC:	0.21 (PhMe/EtOAc 9:1)
MPLC:	PhMe/EtOAc 95:5; 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.78-1.04 (m, 6H), 1.18-1.45 (m, 4H), 1.46-1.73 (m, 4H), 3.17-3.54 (m, 4H), 6.45 (d, J = 14.6Hz, 1H), 6.82 (d, J = 15.6Hz, 1H), 6.98 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 10.4Hz, 1H), 7.36-7.67 (m, 5H).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.0 (t), 31.9 (t), 46.6 (t), 47.9 (t), 122.8 (d), 123.0 (q, <sup>2</sup>J<sub>C-F</sub> = 32.5Hz), 124.0 (q, <sup>1</sup>J<sub>C-F</sub> = 272.1Hz), 125.6 (q, <sup>3</sup>J<sub>C-F</sub> = 3.8Hz, 2C), 127.0 (d, 2C), 129.4 (d), 136.6 (d), 139.9 (q, <sup>4</sup>J<sub>C-F</sub> = 1.5Hz), 141.4 (d), 165.7 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 354.2039, m/z (measured) = 354.2050, difference = 3.11 ppm

GC - MS: short method,  $t_R = 12.04$  min, main fragments 353 (13, M<sup>+</sup>), 310 (10), 225 (100), 128 (33), 86 (13).

#### Compound 13, (2E,4E)-5-(4-acetylphenyl)-N,N-dibutylpenta-2,4-dienamide

Halide:	4-bromoacetophenone (0.38 mmol, 76.5 mg)
Yield:	58% (0.223 mmol, 72.9 mg)
Appearance:	pale green crystals
M.p.:	95-96 °C
TLC:	0.08 (PhMe/EtOAc 9:1) and 0.10 (DCM/MeOH 99:1)
MPLC:	PhMe/EtOAc 90:10, 18g SiO <sub>2</sub> or DCM/MeOH 99:1, 9g

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.81-1.00 (m, 6H), 1.17-1.43 (m, 4H), 1.43-1.73 (m, 4H), 2.55 (s, 3H), 3.20-3.56 (m, 4H), 6.45 (d, J = 14.6Hz, 1H), 6.82 (d, J = 14.5Hz, 1H), 6.90-7.12 (m, 1H), 7.30-7.47 (m, 1H), 7.51 (d, J = 8.4Hz, 2H), 7.89 (d, J = 8.4Hz, 2H)

<sup>13</sup>C-NMR (50 MHz, CDCI<sub>3</sub>):  $\delta$  = 13.83 (q), 13.89 (q), 20.1 (t), 20.3 (t), 26.6 (q), 30.0 (t), 32.0 (t), 46.6 (t), 47.9 (t), 122.8 (d), 126.9 (d), 128.8 (d), 129.6 (d), 136.5 (s), 137.0 (d), 141.0 (s), 141.5 (d), 165.7 (s), 197.3 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 328.2271, m/z (measured) = 328.2265, difference = -1.83 ppm

GC - MS: short method,  $t_R = 14.32$  min, main fragments 327 (15, M<sup>+</sup>), 207 (100), 199 (41), 96 (38), 128 (22).

# Compound 14, (*1E,3E*)-4-(5-(dibutylamino)-5-oxopenta-1,3-dien-1-yl)benzoic acid, ethylester

Halide:	4-bromobenzoic acid, ethyl ester (0.38 mmol, 87.2 mg)
Yield:	37% (0.14mmol, 50.4mg)
Appearance:	yellow crystals
M.p.:	68-70 °C
TLC:	0.17 (LP/EtOAc 5:1)
MPLC:	heptane/EtOAc 12% to 14%; 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.79-1.07 (m, 6H), 1.18-1.42 (m, 4H), 1.38 (t, J = 7.12Hz, 3H) 1.43-1.72 (m, 4H), 3.17-3.51 (m, 4H), 4.36 (q, J = 7.12Hz, 2H) 6.45 (d, J = 14.6Hz, 1H), 6.84 (d, J = 15.5Hz, 1H), 7.00 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 10.4Hz, 1H), 7.46 (dd, J<sub>1</sub> = 14.5Hz, J<sub>2</sub> = 10.3Hz, 1H), 7.49 (d, J = 8.4Hz, 2H), 8.00 (d, J = 8.4Hz, 2H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 14.3 (q), 20.1 (t), 20.3 (t), 30.0 (t), 32.0 (t), 46.6 (t), 47.9 (t), 60.9 (t), 122.5(d), 126.7(d), 129.3(d), 129.9(d), 130.0 (s), 137.2(d), 140.7 (s), 141.6(d), 165.7(s), 166.1(s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 358.2377, m/z (measured) = 358.2366, difference = -3.07 ppm

GC - MS: long method,  $t_R$  = 15.82 min, main fragments 357 (7, M<sup>+</sup>), 229 (55), 129 (52), 128 (100), 127 (38).

## Compound 15, (2E,4E)-N,N-dibutyl-5-(4-cyanophenyl)penta-2,4-dienamide

Halide:	4-bromobenzonitrile (0.38 mmol, 70.0 mg)
Yield:	67% (0.258 mmol, 80.1 mg)
Appearance:	off-white crystals
M.p.:	86-88°C
TLC:	0.21 (PhMe/EtOAc 9:1)
MPLC:	PhMe/EtOAc 95:5; 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.79-1.07 (m, 6H), 1.18-1.42 (m, 4H), 1.43-1.72 (m, 4H), 3.17-3.51 (m, 4H), 6.47 (d, J = 14.6Hz, 1H), 6.77 (d, J = 15.5Hz, 1H), 6.99 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 10.8Hz, 1H), 7.31-7.66 (m, 5H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.0 (t), 32.0 (t), 46.6 (t), 47.9 (t), 111.4 (s), 118.8 (s), 123.6(d), 127.2 (d), 130.5 (d), 132.4 (d), 136.1(d), 140.1 (t), 141.0 (d), 165.5 (t)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 311.2118, m/z (measured) = 311.2119, difference = 0.32 ppm

GC - MS: short method,  $t_R = 14.60$  min, main fragments 310 (10, M<sup>+</sup>), 207 (10), 182 (100), 127 (55), 86 (9).

#### Compound 16, (2E,4E)-N,N-dibutyl-5-(thiophen-3-yl)penta-2,4-dienamide

After 1 h reaction time, the starting material was not fully converted. Another 2.0 mol % catalyst were added. After a total reaction time of 44h GC-MS indicated no further progress of the reaction.

Halide:	3-bromothiophen (0.39 mmol, 63.8 mg)
Yield:	18% (0.07 mmol, 19.9 mg)
MPLC:	heptane/EtOAc 10:1, 18g SiO <sub>2</sub>

Alternative procedure: (*E*)-penta-2,4-dienoic dibutylamide (1.00 equiv., 79.5 mg, 0.38 mmol), 3bromothiophene (1.97equiv., 122.0 mg, 0.75 mmol), Pd $(OAc)_2$  (2 mol %, 1.75 mg), dppp (2.0 mol %, 3.18 mg) and K<sub>2</sub>CO<sub>3</sub> (3.0 equiv., 159.6 mg, 1.15 mmol) were added in a vial and diluted in 2.6 ml degassed DMF under argon and heated at 100 °C for 16h. GC-MS indicated only partial conversion of the starting materials. The reaction mixture was extracted with diethylether. The organic phase was washed with brine and dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of the solvent the pure product was isolated by column chromatography. Additionally, 36 % (28.3 mg) (E)-penta-2,4-dienoic dibutylamide could be recovered.

Yield:	35% (0.13mmol, 38.3 mg)
Appearance:	yellow solid
M.p.:	83-84 °C
TLC:	0.23 (LP/EtOAc 5:1)
MPLC:	LP/EtOAc 8:1, 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.84-1.07 (m, 6H), 1.20-1.46 (m, 4H), 1.44-1.70 (m, 4H), 3.22-3.47 (m, 4H), 6.36 (d, J = 14.6Hz, 1H), 6.73 (dd, J<sub>1</sub> = 15.5Hz, J<sub>2</sub> = 10.1Hz, 1H), 6.87 (d, J = 15.4Hz, 1H), 7.43 (dd, J<sub>1</sub> = 14.7Hz, J<sub>2</sub> = 10.3Hz, 1H), 7.23-7.32 (m, 3H)

<sup>13</sup>C-NMR (50 MHz, CDCI<sub>3</sub>):  $\delta$  = 13.8 (q), 13.9 (q), 20.1 (t), 20.3 (t), 30.1 (t), 31.9 (t), 46.6 (t), 48.0 (t), 120.6 (d), 124.3 (d), 126.4 (d), 127.0 (d), 132.5 (d), 139.5 (t), 142.4(d), 166.1 (t)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 292.1730, m/z (measured) = 292.1723, difference = -2.40 ppm

GC - MS: long method,  $t_R$  = 12.11 min, main fragments 291 (3, M<sup>+</sup>), 163 (100), 135 (52), 134 (36), 91 (67)

#### Compound 17, (2E,4E)-N,N-dibutyl-5-(pyridin-4-yl)penta-2,4-dienamide

After 1h reaction time, the starting material was not fully converted. Another 2.0 mol % catalyst were added and the solution was heated over night. According to GC-MS the halide was comsumed. However, a significant amount of the diene coupling partnter was still present. Therefore another equivalent of 1-bromo-4-fluorobenzen was added and the reaction was heated for a total of 44h.

Halide:	4-bromopyridin (0.76 mmol, 120.2 mg)
Yield:	59% (0.22 mmol, 64.3 mg)
Appearance:	yellow crystals
TLC:	0.14 (LP/EtOAc 17:3)
MPLC:	heptane/EtOAc 10:1, 18g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.87-1.01 (m, 6H), 1.22-1.44 (m, 4H), 1.44-1.71 (m, 4H), 3.24-3.50 (m, 4H), 6.49 (d, J = 14.7Hz, 1H), 6.75 (d, J = 15.6Hz, 1H), 7.07 (dd, J<sub>1</sub> = 15.4Hz, J<sub>2</sub> = 11.0Hz, 1H), 7.29 (d, J = 6.0Hz, 2H), 7.45 (dd, J<sub>1</sub> = 14.6Hz, J<sub>2</sub> = 11.0Hz, 1H), 8.57 (d, J = 5.5Hz, 2H)

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.82 (q), 13.89 (q), 20.1 (t), 20.3 (t), 30.0 (t), 32.0 (t), 46.6 (t), 48.0 (t), 121.0 (d), 123.9 (d), 131.3 (d), 135.4 (d), 140.9 (d), 143.7 (s) 150.3 (d), 165.5 (s)

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 287.2118, m/z (measured) = 287.2109, difference = -3.13 ppm

GC - MS: long method,  $t_R$  = 12.30 min, main fragments 286 (4, M<sup>+</sup>), 158 (100), 130 (46), 103 (23), 77 (24)

#### Compound 18, (2E,4E)-N,N-dibutyl-5-(pyridin-3-y)penta-2,4-dienamide

Halide:	3-bromopyridine (0.40 mmol, 62.5 mg)
Yield:	62% (0.239 mmol, 68.5 mg)
Appearance:	orange oil
TLC:	0.24 (DCM/MeOH 97:3)
MPLC:	DCM/MeOH 97:3; 9g SiO <sub>2</sub>

<sup>1</sup>H-NMR (200 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.80-1.00 (m, 6H), 1.21-1.42 (m, 4H), 1.43-1.73 (m, 4H), 3.17-3.57 (m, 4H), 6.47 (d, J = 14.7Hz, 1H), 6.80 (d, J = 15.7Hz, 1H), 6.99 (dd, J<sub>1</sub> = 15.6Hz, J<sub>2</sub> = 10.5Hz, 1H), 7.15-7.38 (m, 1H), 7.47 (dd, J<sub>1</sub> = 14.6Hz, J<sub>2</sub> = 10.5Hz, 1H), 7.76 (td, J<sub>1</sub> = 8.0Hz, J<sub>2</sub> = 1.9Hz, 1H), 8.49 (dd, J<sub>1</sub> = 4.8Hz, J<sub>2</sub> = 1.4Hz, 1H), 8.68 (d, J = 1.8Hz, 1H).

<sup>13</sup>C-NMR (50 MHz, CDCl<sub>3</sub>):  $\delta$  = 13.8 (q), 20.1 (t), 20.3 (t), 30.0 (t), 31.9 (t), 46.6 (t), 47.9 (t), 122.5 (d), 123.5 (d), 129.0 (d), 132.1 (s), 133.2 (d), 134.5 (d), 141.4 (d), 148.6 (d), 149.1 (d), 165.7 (s).

HR-MS: [M+H]<sup>+</sup> m/z (predicted) = 287.2118, m/z (measured) = 287.2112, difference = -2.09 ppm

GC - MS: short method,  $t_R = 12.69$  min, main fragments 286 (7, M<sup>+</sup>), 206 (8), 158 (100), 131 (80), 77 (35).

#### **Biological Activity**

# Expression of $GABA_A$ and TRPV1 channels in Xenopus laevis oocytes and two-microelectrode voltage-clamp experiments

Preparation of stage V–VI oocytes from Xenopus laevis and synthesis of capped off run-off poly(A<sup>+</sup>) rat cRNA transcripts from linearized cDNA templates (pCMV vector) was performed as previously described (Schöffmann et al., 2014). Female Xenopus laevis frogs (NASCO, Fort Atkinson, USA) were anesthetized by 15 min incubation in a 0.2% MS-222 (methane sulfonate salt of 3-aminobenzoic acid ethyl ester; Sigma Aldrich, Vienna, Austria) solution before removal of parts of the ovaries. Follicle membranes from isolated oocytes were enzymatically digested with 2 mg/mL collagenase (Type 1A, Sigma-Aldrich, Vienna, Austria). Selected stage V-VI oocytes were injected with about 10-50 nl of DEPC- treated water (diethyl pyrocarbonate, Sigma, Germany) containing the different cRNAs (the rat TRPV1 clone was kindly provided by Prof. David Julius (Department of Cellular and Molecular Pharmacology, University of California, San Francisco) at a concentration of approximately 150 - 2000 pg/nl. The amount of cRNA was determined by means of a NanoDrop ND-1000 (Kisker-biotech, Steinfurt, Germany). To ensure expression of the  $\gamma_{2s}$ -subunit in GABA<sub>A</sub> receptors, cRNAs for expression of  $\alpha_1\beta_2\gamma_{25}$  receptors were mixed in a ratio of 1:1:10 (Boileau et al., 2002) After injection, oocytes were stored at 18°C for 24-48h in ND96 solution (90 mM NaCl, 1 mM KCl, 1 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 1 mM CaCl<sub>2</sub> and 5 mM HEPES; pH 7.4) containing penicillin G (10,000IU/100ml) and streptomycin (10mg/100ml). Electrophysiological experiments on GABA<sub>A</sub> receptors and TRPV1 channels were performed using the two-microelectrode-voltage-clamp method at a holding potential of -70 mV (GABA<sub>A</sub> receptors) and -60mV (TRPV1), respectively, making use of a TURBO TEC 01C amplifier (npi electronic, Tamm, Germany) and an Axon Digidata 1322A interface (Molecular Devices, Sunnyvale, CA). Microelectrodes were filled with 2M KCl and had resistances between 1 and  $3M\Omega$ . Data acquisition was done using pCLAMP v.9.2.

#### **Fast Perfusion System**

Compounds were applied by means of the ScreeningTool (npi electronic, Tamm, Germany) fast perfusion system as previously described<sup>3</sup>. To elicit chloride currents through  $\alpha_1\beta_2\gamma_{25}$  receptors (I<sub>GABA</sub>), the chamber was perfused with 120 µL of GABA or GABA+compound-containing, while cationic currents through TRPV1 channels (I<sub>Capsaicin</sub>) were elicited by perfusion of the chamber with 120µl capsaicin or capsaicin+compound containing solution, respectively, at a volume rate of 300 µL/s (Baburin et al., 2006).

After co-application of GABA+compound (GABA<sub>A</sub> receptors) or capsaicin+compound (TRPV1 channels) containing solution a 15min washout period was applied to exclude possible slow recovery from desensitization. Oocytes with maximal current amplitudes >3  $\mu$ A were discarded to exclude voltage clamp errors.

#### **Data Analysis**

Modulation of chloride currents through GABA<sub>A</sub> receptors ( $I_{GABA}$ ) was studied by co-application of GABA (at concentrations eliciting between 3% and 7% of the maximal current amplitude (EC<sub>3-7</sub>)) and the respective derivative at a concentration of 100µM. The GABA EC<sub>3-7</sub> was determined for each oocyte individually. Modulation of the chloride currents was defined as ( $I_{(GABA+Comp)}/I_{GABA}$ ) - 1, where

 $I_{\text{(GABA+Comp)}}/\text{represents}$  the current response in the presence of a given compound and  $I_{\text{GABA}}$  is the control GABA current.

Modulation of currents through TRPV1 channels was studied by co-application of 1  $\mu$ M capsaicin (I<sub>Capsaicin</sub>) and the respective compound at a concentration of 100 $\mu$ M. Modulation of cationic currents through TRPV1 channels was defined as (I<sub>(capsaicin+comp)</sub>/I<sub>capsaicin</sub>) – 1, where I<sub>(capsaicin+comp)</sub> represents the current response in the presence of a given compound and I<sub>Capsaicin</sub> is the control current elicited by application of 1  $\mu$ M capsaicin.

Data analysis was performed using Origin Software (OriginLab Corporation, USA). Each data point represents the mean $\pm$ SE from at least 3 oocytes and  $\geq$  2 oocyte batches. Statistical significance was calculated by ANOVA followed by a *Dunnet* post-hoc mean comparison. *p* values <0.05 were considered as statistically significant.

- 1 K. Sparrow, D. Barker and M. A. Brimble, *Tetrahedron*, 2011, **67**, 7989.
- 2 M. Abbari, P. Cosquer, F. Tonnard, Y. Y. C. Y. L. Ko and R. Carrie, *Tetrahedron*, 1991, **47**, 71.
- 3 S. Khom, I. Baburin, E. N. Timin, A. Hohaus, W. Sieghart and S. Hering, *Molecular Pharmacology*, 2006, **69**, 640.