Supplementary Information

# ω-Heteroarylalkylcarbamates as inhibitors of fatty acid amide hydrolase (FAAH)

*Tobias Terwege, Helmut Dahlhaus, Walburga Hanekamp, Matthias Lehr**

Institute of Pharmaceutical and Medicinal Chemistry, University of Münster,
Corrensstrasse 48, 48149 Münster, Germany

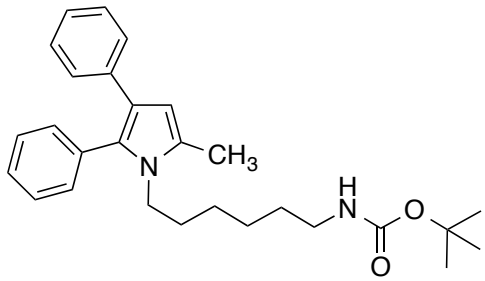
* Corresponding author. Tel.: +49251 83 33331, Fax: +49251 83 32144,
E-mail: lehrm@uni-muenster.de

**Supplementary Information content:**

## 1. Synthesis of the target compounds

**General** Column chromatography was performed on silica gel 60, particle size 0.040-0.063 mm, from Macherey & Nagel. Melting points were determined on a Büchi B-540 apparatus and are uncorrected. $^{1}$H-NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer (400 MHz), a Varian Unity Plus 600 spectrometer (600 MHz) or an Agilent VNMRS-600 spectrometer (600 MHz). $^{13}$C-NMR spectra were measured on a Varian Mercury Plus 400 spectrometer (101 MHz) or an Agilent VNMRS-600 spectrometer (151 MHz). Electron ionization (EI) mass spectra were obtained on a Finnigan GCQ apparatus. The high resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q II spectrometer using electro spray chemical ionization (ESI) or atmospheric pressure chemical ionization (APCI). The purity of the target compounds was assessed by reversed phase HPLC on a Nucleosil 100 RP 18 3 µm column (3 mm inside diameter x 125 mm) with a gradient consisting of acetonitrile/water/trifluoroacetic acid (42:58:0.1 to 86:14:0.1, v/v/v) at a flow rate of 0.40 mL/min or on a Phenomenex Aqua C18 3 µm columns (4.6 mm inside diameter x 75 mm) eluting isocratically with acetonitrile/water/phosphoric acid (85%) (80:20:0.1, v/v/v) at a flow rate of 0.70 mL/min. UV-absorbance was measured at 254 nm. Purities of the target compounds were greater or equal 95%.

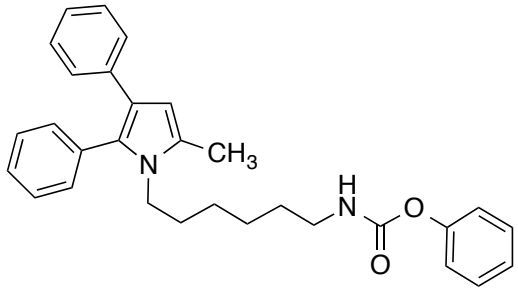
***tert*-Butyl *N*-[6-(5-methyl-2,3-diphenylpyrrol-1-yl)hexyl]carbamate (8)**




A solution of 5-methyl-2,3-diphenylpyrrole (**7**) (0.21 g, 0.90 mmol) in dry DMSO (5 mL) was treated with K-*tert*-butylate (0.12 g, 1.07 mmol) and heated at 80 °C for 15 min. Then a *tert*-butyl (6-bromohexyl)carbamate (0.26 g, 0.93 mmol) in dry DMSO (5 mL) was added and the mixture was heated at 90 °C for 3 h. The cooled reaction mixture was poured into water and exhaustively extracted with ethyl acetate. The combined organic layers were washed three times with water and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 19:1) to afford **8** as an oil (0.21 g, 54%). $C_{28}H_{36}N_2O_2$ (432.61); $^{1}$H-NMR (400 MHz, $CDCl_3$): δ 1.02 – 1.09 (m, 4H), 1.21 – 1.28 (m, 2H), 1.37 (s, 9H), 1.38-1.45 (m, 2H), 2.26 (s, 3H), 2.89 – 2. 98 (m, 2H), 3.61 – 3.67 (m, 2H), 4.34 (s, 1H), 6.11 (s, 1H), 6.92 – 7.32 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 26.1, 26.2,

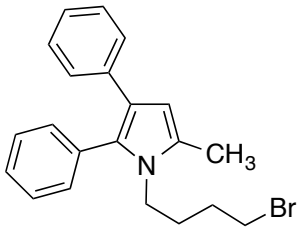
28.4, 29.8, 30.8, 40.4, 43.8, 106.8, 122.5, 124.7, 127.4, 127.5, 127.9, 128.5, 128.5, 129.5, 131.4, 133.8, 136.6, 155.9; MS (EI): *m/z* (%) 432.2 (80) [$M^+$], 376.1 (40), 246.1 (100).

**Phenyl *N*-6-(5-methyl-2,3-diphenylpyrrol-1-yl)hexylcarbamate (9)**



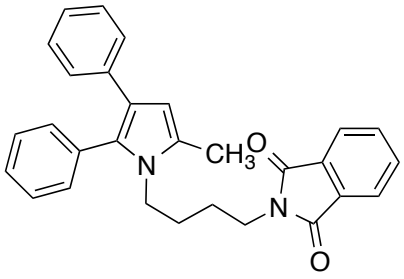

To solution of **8** (0.18 g, 0.42 mmol) in dry $CH_2Cl_2$ (20 mL) was added trifluoroacetic acid (5.7 mL) at 0 °C. After stirring at room temperature for 4 h, the mixture was concentrated under reduced pressure. The residue was treated repeatedly with hexane, and the solvent was evaporated each time. Then water was added, the pH adjusted to about 9 with 10% aqueous NaOH, and the mixture exhaustively extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. An aliquot of the residue consisting of 6-(5-methyl-2,3-diphenylpyrrol-1-yl)-hexan-1-amine (0.070 g, 0.21 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL) and treated with triethylamine (0.021 g, 0.21 mmol) and phenyl chloroformate (0.033 g, 0.21 mmol). After stirring at room temperature for 3 h, the mixture was poured into water, acidified with dilute HCl, and exhaustively extracted with ethyl acetate. The combined organic phases were washed with half-concentrated brine and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to afford **9** as an oil (0.060 g). $C_{30}H_{32}N_2O_2$ (452.60); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.12 – 1.22 (m, 4H), 1.37 – 1.46 (m, 2H), 1.46 – 1.55 (m, 2H), 2.34 (s, 3H), 3.16 (q, $J$ = 6.8 Hz, 2H), 3.71 – 3.77 (m, 2H), 4.92 (s, 1H), 6.19 (s, 1H), 7.00 – 7.41 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 26.0, 26.2, 29.5, 30.7, 41.0, 43.7, 106.8, 121.5, 124.7, 125.2, 127.4, 127.5, 127.9, 128.5, 128.5, 129.2, 129.5, 131.4, 133.8, 136.6, 151.0, 154.5; MS (EI): *m/z* (%) 452.3 (2)[$M^+$], 358.2 (56), 246.1 (100). HRMS-ESI [$M$+H]$^+$ calculated: 453.2537, found: 453.2545.

**1-(4-Bromobutyl)-5-methyl-2,3-diphenylpyrrole (11)**




A mixture of 5-methyl-2,3-diphenylpyrrole (**10**) (0.23 g, 0.99 mmol), 1,4-dibromobutane (2.17 g, 10.1 mmol), tetrabutylammonium bromide (0.10 g, 0.31 mmol) and toluene (10 mL) was treated with a 50% (m/m) aqueous NaOH solution (2 g). The mixture was stirred at 45 °C for 2 h, poured into water, and extracted exhaustively with ethyl acetate. The combined organic phases were washed with half-concentrated brine and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 49:1) to afford **11** as an oil (0.23 g, 63%). $C_{21}H_{22}BrN$ (368.32); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.63 – 1.68 (m, 4H), 2.35 (s, 3H), 3.17 – 3.23 (m, 2H), 3.75 – 3.81 (m, 2H), 6.19 (s, 1H), 7.01 – 7.40 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 29.4, 29.7, 32.8, 43.0, 107.1, 121.7, 124.7, 127.5, 127.5, 127.9, 128.5, 128.6, 129.6, 131.6, 133.6, 136.5; MS (EI): *m/z* (%) 367.0 (19) [$M^+$], 288.2 (100), 246.1 (24).

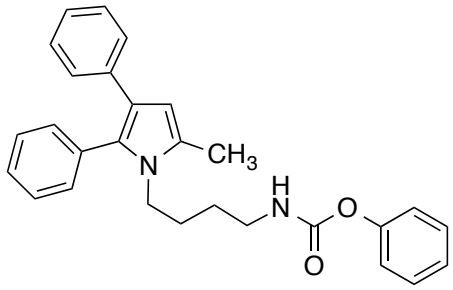
**2-[4-(5-Methyl-2,3-diphenylpyrrol-1-yl)butyl]isoindoline-1,3-dione (12)**




A solution of **11** (0.21 g, 0.57 mmol) and potassium phthalimide (0.21 g, 1.13 mmol) in DMF (10 mL) was heated with stirring at 130°C for 2 h. The cooled reaction mixture was poured into water and exhaustively extracted with ethyl acetate. The combined organic phases were washed with half-concentrated brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to afford **12** as an oil (0.21 g, 85%). $C_{29}H_{26}N_2O_2$ (434.54); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.46 – 1.53 (m, 4H), 2.32 (s, 3H), 3.48 – 3.53 (m, 2H), 3.74 – 3.81 (m, 2H), 6.16 (s, 1H), 6.99 – 7.31 (m, 10H), 7.71 (dd, $J$ = 5.5 Hz and 3.1 Hz, 2H), 7.82 (dd, $J$ = 5.5 Hz and 3.1 Hz, 2H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 25.6, 28.2, 37.2, 43.4, 106.9, 121.6, 123.2, 123.6 (Pyr), 124.7, 127.5, 127.5, 127.9, 128.5, 128.5,

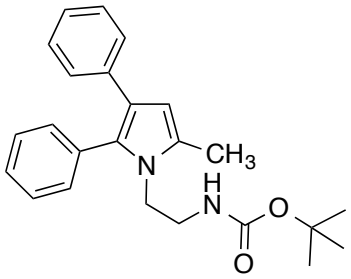
129.5, 131.2, 132.0, 133.6, 133.9, 136.5, 168.2; MS (EI): *m/z* (%) 434.1 (87) [$M^+$], 246.1 (100).

**Phenyl *N*-[4-(5-methyl-2,3-diphenylpyrrol-1-yl)butyl]carbamate (13)**



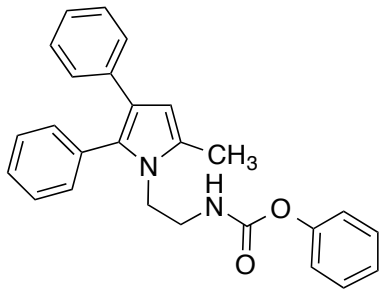

To a solution of **12** (0.19 g, 0.44 mmol) in ethanol (15 mL) was added aqueous hydrazine-hydrate solution (24%) (0.91 g, 4.4 mmol) and the mixture was heated under reflux for 3 h. After distilling off most of the ethanol, the reaction mixture was treated with $CHCl_3$ (30 mL) and 10% aqueous NaOH solution, and extracted exhaustively with $CHCl_3$. The combined organic phases dried over anhydrous sodium sulfate and concentrated. An aliquot of the residue consisting of 4-(5-methyl-2,3-diphenylpyrrol-1-yl)butyl-1-amine (0.070 g, 0.23 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL) and treated with triethylamine (0.030 g, 0.30 mmol) and phenyl chloroformate (0.040 g, 0.26 mmol). After stirring at room temperature for 2 h, the mixture was poured into water, acidified with dilute HCl, and exhaustively extracted with ethyl acetate. The combined organic phases were washed with half-concentrated brine and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to afford **13** as an oil (0.080 g). $C_{28}H_{28}N_2O_2$ (424.54); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.33 – 1.43 (m, 2H), 1.48 – 1.61 (m, 2H), 2.35 (s, 3H), 3.02 – 3.13 (m, 2H), 3.77 – 3.84 (m, 2H), 4.74 – 4.83 (m, 1H), 6.20 (s, 1H), 7.01 – 7.43 (m, 15H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 26.7, 27.9, 40.4, 43.3, 107.0, 121.5, 121.7, 124.8, 125.3, 127.5, 127.6, 127.9, 128.6, 128.6, 129.3, 129.4, 131.3, 133.8, 136.5, 150.9, 154.5; MS (EI): *m/z* (%) 424.0 (2) [$M^+$], 330.1 (99), 246.2 (100), 234.2 (35), 170.2 (34).

***tert*-Butyl *N*-[2-(5-methyl-2,3-diphenylpyrrol-1-yl)ethyl]carbamate (14)**



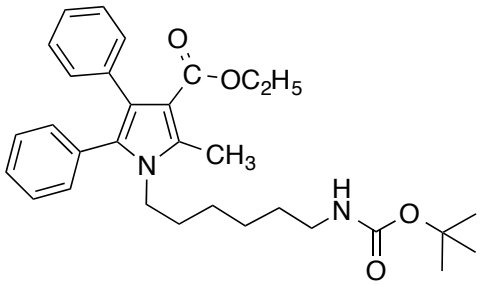
Following the procedure described for the synthesis of **8**, 5-methyl-2,3-diphenylpyrrole (**7**) (0.21 g, 0.90 mmol) was reacted with *tert*-butyl *N*-(2-bromoethyl)carbamate (0.20 g, 0.90 mmol) to yield **14** as a solid (0.15 g, 44%). $C_{24}H_{28}N_2O_2$ (376.50); mp 124 – 127 °C; $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.40 (s, 9H), 2.36 (s, 3H), 3.11 (q, $J$ = 6.3 Hz, 2H), 3.91 (t, $J$ = 6.2 Hz, 2H), 4.42 (s, 1H), 6.19 (s, 1H), 7.02 – 7.41 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 28.3, 40.8, 43.5, 107.2, 122.1, 124.8, 127.5, 127.7, 127.9, 128.7, 129.3, 129.4, 131.3, 133.5, 136.4, 155.6; MS (EI): *m/z* (%) 376.1 (88) [$M^+$], 320.1 (79), 246.2 (100).

**Phenyl *N*-[2-(5-methyl-2,3-diphenylpyrrol-1-yl)ethyl]carbamate (15)**



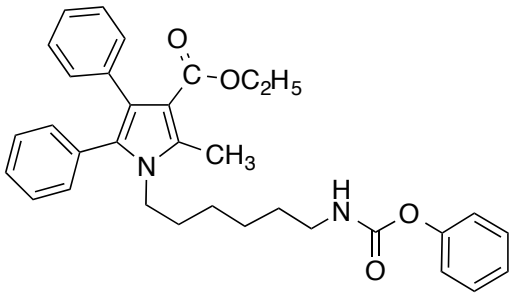
Compound **15** was obtained from **14** (0.10 g, 0.27 mmol) in a similar manner as described for the synthesis of **9**. Yield: 0.030 g. $C_{26}H_{24}N_2O_2$, (396.49); mp 147 – 148 °C; $^1$H-NMR (400 MHz, $CDCl_3$): δ 2.40 (s, 3H), 3.26 (q, $J$ = 6.4 Hz, 2H), 4.02 (t, $J$ = 6.4 Hz, 2H), 4.86 – 4.92 (m, 1H), 6.22 (s, 1H), 7.01 – 7.44 (m, 15H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.5, 41.4, 43.1, 107.5, 121.5, 122.3, 124.9, 125.4, 127.5, 127.8, 128.0, 128.8, 129.3, 129.5, 131.3, 133.4, 136.3, 150.8, 154.5; MS (EI): *m/z* (%) 396.0 (23) [$M^+$], 302.0 (72), 246.0 (100), 230.1 (41), 95.1 (38). HRMS-ESI [$M$+H]$^+$ calculated: 397.1911, found: 397.1918.

**Ethyl 1-[6-(*tert*-butoxycarbonylamino)hexyl]-2-methyl-4,5-diphenylpyrrole-3-carboxylate (17)**



A solution of ethyl 2-methyl-4,5-diphenylpyrrole-3-carboxylate (**16**) (0.15 g, 0.49 mmol) in dry DMSO (10 mL) was treated with K-*tert*-butylate (0.090 g, 0.80 mmol) and heated at 60 °C for 15 min. Then a solution of *tert*-butyl *N*-(6-bromohexyl)carbamate (0.14 g, 0.50 mmol) in dry DMSO (5 mL) was added and the mixture was heated at 60 °C for 3 h. The cooled reaction mixture was poured into water and exhaustively extracted with ethyl acetate. The combined organic layers were washed three times with water and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 19:1) to afford **17** as an oil (0.11 g, 44%). $C_{31}H_{40}N_2O_4$ (504.67); $^1$H-NMR (400 MHz, $CDCl_3$): δ 0.99 (t, $J$ = 7.1 Hz, 3H), 1.06 – 1.17 (m, 4H), 1.27 – 1.36 (m, 2H), 1.43 (s, 9H), 1.46 – 1.55 (m, 2H), 2.61 (s, 3H), 2.95 – 3.05 (m, 2H), 3.73 – 3.80 (m, 2H), 4.06 (q, $J$ = 7.1 Hz, 2H), 4.42 (s, 1H), 7.05 – 7.28 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 11.7, 13.9, 26.0, 26.1, 28.4, 29.8, 30.5, 40.3, 44.0, 59.2, 111.2, 123.7, 125.5, 126.9, 127.5, 128.1, 130.7, 131.2, 131.4, 132.2, 135.0, 135.9,155.9, 166.0; MS (EI): *m/z* (%) 504.1 (81) [$M^+$], 448.1 (54), 402.1 (67), 343.1 (100).

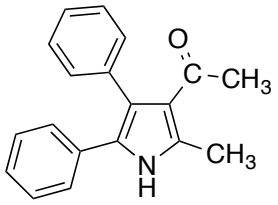
**Ethyl 2-methyl-1-[6-(phenoxycarbonylamino)hexyl]-4,5-diphenylpyrrole-3-carboxylate (18)**



Compound **18** was obtained from **17** (0.090 g, 0.18 mmol) in a similar manner as described for the synthesis of **9**. Yield: 0.070 g. $C_{33}H_{36}N_2O_4$ (524.66); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.00 (t, $J$ = 7.1 Hz, 3H), 1.11 – 1.21 (m, 4H), 1.48 – 1.56 (m, 2H), 1.37 – 1.46 (m, 2H), 2.62 (s, 3H), 3.16 (dt, $J$ = 6.7 Hz, 2H), 3.76 – 3.83 (m, 2H), 4.07 (q,

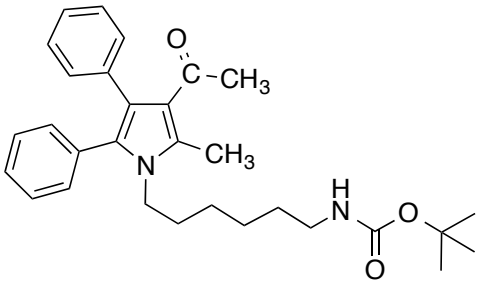
*J* = 7.1 Hz, 2H), 4.88 – 4.98 (m, 1H), 7.05 – 7.36 (m, 15H); MS (EI): *m/z* (%) 523.9 (3) [$M^+$], 430.0 (100), 272.1 (45), 246.1 (76), 149.0 (36), 94.1 (38), 65 (38).

**3-Acetyl-2-methyl-4,5-diphenylpyrrole (19)**




A suspension of $AlCl_3$ (0.45 g, 3.37 mmol) in dry $CH_2Cl_2$ (5 mL) was treated dropwise at 0°C with acetyl chloride (0.15 g, 1.91 mmol). After being stirred at 0°C for 30 min, the mixture was added dropwise at the same temperature to a solution of 5-methyl-2,3-diphenylpyrrole (**10**) (0.42 g, 1.80 mmol) in dry $CH_2Cl_2$ (10 mL) and allowed to warm up to room temperature overnight. The reaction mixture was poured into water and extracted exhaustively with $CH_2Cl_2$. The combined organic phases were washed with half-concentrated brine and with brine, dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to afford **19** as a solid (0.12 g, 24%). $C_{19}H_{17}NO$ (275.35); mp 178 – 180 °C; $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.87 (s, 3H), 2.60 (s, 3H), 7.08 – 7.38 (m, 10H), 8.48 (s, 1H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 14.4, 30.9, 122.8, 122.9, 126.6, 126.6, 126.9, 127.1, 128.4, 128.5, 130.9, 132.2, 135.0 136.5, 197.1; MS (EI): *m/z* (%) 275.1 (79) [$M^+$], 260.1 (100), 245.1 (26).

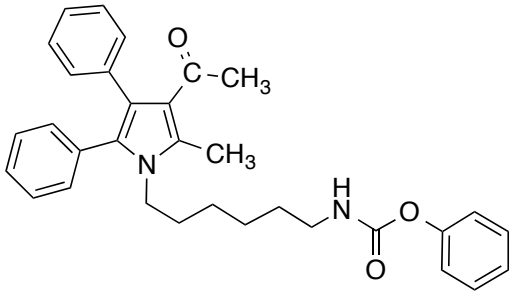
***tert*-Butyl *N*-[6-(3-acetyl-2-methyl-4,5-diphenylpyrrol-1-yl)hexyl]carbamate (20)**




Compound **19** (0.15 g, 0.54 mmol) was reacted with *tert*-butyl *N*-(6-bromohexyl)carbamate (0.15 g, 0.54 mmol) in a similar manner as described for the synthesis of **8** to yield **20** as an oil (0.21 g, 81%). $C_{30}H_{38}N_2O_3$ (474.64), $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.06 – 1.17 (m, 4H), 1.26 – 1.37 (m, 2H), 1.43 (s, 9H), 1.46 – 1.56 (m, 2H), 1.89 (s, 3H), 2.56 (s, 3H), 2.92 – 3.07 (m, 2H), 3.73 – 3.80 (m, 2H), 4.42 (s, 1H), 7.07 – 7.31 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 12.0, 26.0, 26.2, 28.4, 29.8, 30.4, 31.1, 40.3, 43.8, 121.9, 123.6, 126.2, 127.6, 127.7, 128.1, 130.7, 131.0, 131.4, 132.0, 134.1, 136.3,

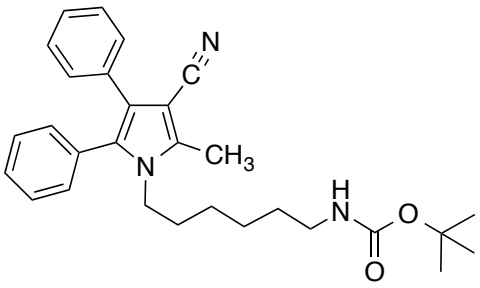
197.7; MS (EI): *m/z* (%) 474.0 (18) [$M^+$], 418.0 (40), 375.0 (100), 331.0 (49), 246.2 (37), 234.1 (37), 95.1 (23).

**Phenyl *N*-[6-(3-acetyl-2-methyl-4,5-diphenylpyrrol-1-yl)hexyl]carbamate (21)**



Compound **21** was obtained from **20** (0.19 g, 0.40 mmol) in a similar manner as described for the synthesis of **9**. Yield: 0.16 g. An aliquot of the crude product (0.030 g) was dissolved in a small amount of DMSO and was cleaned up by reversed phase HPLC on a semi-preparative Kromasil 100 C18 5 $\mu$m column (10 mm I.D. x 250 mm) protected with an analogously filled guard column (10 mm I.D. x 50 mm). Elution was performed with acetonitrile/water (80/20, v/v) at a flow rate of 3 mL/min and the effluent was monitored at 254 nm. Pure compound **21** was obtained as solid after distilling off the organic solvent and freeze-drying the remaining aqueous phase (0.010 g). $C_{32}H_{34}N_2O_3$ (494.64); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.10 – 1.21 (m, 4H), 1.36 – 1.46 (m, 2H), 1.47 – 1.57 (m, 2H), 1.89 (s, 3H), 2.59 (s, 3H), 3.16 (dt, $J$ = 6.8 Hz, 2H), 3.75 – 3.82 (m, 2H), 4.91 – 4.98 (m, 1H), 7.05 – 7.36 (m, 15H); MS (EI): *m/z* (%) 494.1 (2) [$M^+$], 469.7 (100), 399.8 (63), 246.1 (64). HRMS-ESI [$M$+H]$^+$ calculated: 495.2642, found: 495.2650.

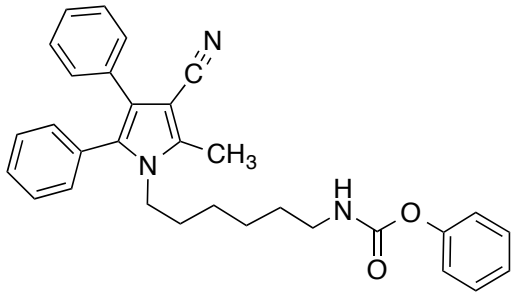
***tert*-Butyl *N*-[6-(3-cyano-2-methyl-4,5-diphenylpyrrol-1-yl)hexyl]carbamate (23)**



2-Methyl-4,5-diphenylpyrrole-3-carbonitrile (**22**) (0.207 g, 0.80 mmol) was reacted with *tert*-butyl *N*-(6-bromohexyl)carbamate (0.224 g, 0.80 mmol) in a similar manner as described for the synthesis of **8** to yield **23** as an oil (0.22 g, 60%). $C_{29}H_{35}N_3O_2$ (457.61); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.05 – 1.18 (m, 4H), 1.27 – 1.36 (m, 2H), 1.43 (s, 9H), 1.43 – 1.53 (m, 2H), 2.49 (s, 3H), 2.94 – 3.06 (m, 2H), 3.71 – 3.78 (m, 2H), 4.40 (s, 1H), 7.10 – 7.41 (m, 10H); $^{13}$C-NMR (100 MHz, $CDCl_3$): δ 11.8, 26.0, 26.1,

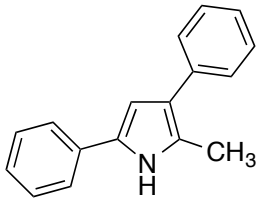
28.4, 29.7, 30.3, 40.2, 44.6, 79.1, 91.8, 117.4, 123.5, 126.3, 128.1, 128.3, 128.7, 128.8, 130.7, 131.2, 131.4, 133.0, 137.8, 155.9; MS (EI): *m/z* (%) 456.8 (9) [$M^+$], 401.0 (100), 342.1 (55), 271.1 (98), 259.1 (33).

**Phenyl *N*-[6-(3-cyano-2-methyl-4,5-diphenylpyrrol-1-yl)hexyl]carbamate (24)**



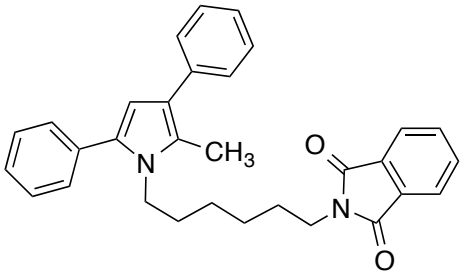

Compound **24** was obtained from **23** (0.20 g, 0.44 mmol) in a similar manner as described for the synthesis of **9**. Yield: 0.18 g. $C_{31}H_{31}N_3O_2$ (477.61); $^1$H-NMR (400 MHz, $CDCl_3$): δ 1.11 – 1.21 (m, 4H), 1.36 – 1.46 (m, 2H), 1.46 – 1.55 (m, 2H), 2.49 (s, 3H), 3.16 (dt, $J$ = 6.7 Hz, 2H), 3.74 – 3.81 (m, 2H), 4.91 – 4.97 (m, 1H), 7.08 – 7.41 (m, 15H); MS (EI): *m/z* (%) 477.0 (6) [$M^+$], 453.0 (18), 383.1 (61), 271.1 (100), 94.1 (56).

**2-Methyl-3,5-diphenylpyrrole (25)**



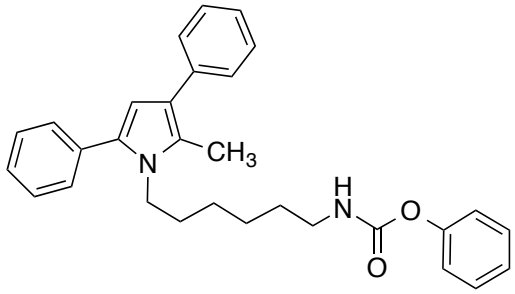

A suspension of 1,3-diphenylpentane-1,4-dione (300 mg, 1.19 mmol) and ammonium acetate (1.7 g, 22 mmol) in ethanol (30 mL) was refluxed for 1 h. The reaction mixture was poured into half-saturated brine (30 mL) and extracted with dichloromethane (3x 40 mL). The organic extracts were combined and washed with brine, dried over anhydrous sodium sulfate, and concentrated, yielding **25** as a solid (276 mg, 99%). $C_{17}H_{15}N$ (233.31); mp 53 – 55°C, $^1$H NMR (400 MHz, $CDCl_3$): δ 2.48 (s, 3H), 6.63 (d, $J$ = 2.9 Hz, 1H), 7.17-7.25 (m, 2H), 7.34-7.42 (m, 4H), 7.44-7.51 (m, 4H), 8.18 ppm (s, 1H); MS (EI): *m/z* (%) 233.0 (100) [$M^+$].

**2-[6-(2-Methyl-3,5-diphenylpyrrol-1-yl)hexyl]isoindoline-1,3-dione (26)**



To a solution of **25** (230 mg, 0.99 mmol) in dry DMSO (20 mL) was added under nitrogen K-*tert*-butylate (121 mg, 1.08 mmol). The mixture was stirred at 50 °C for 15 min. Then a solution of *N*-(6-bromohexyl)phthalimide (382 mg, 1.23 mmol) in dry DMSO (2 mL) was added dropwise over 1 h and the solution was stirred at 50 °C for an additional 1 h. The reaction mixture was cooled, poured into half-saturated brine and extracted with dichloromethane. The organic extracts were combined and washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) yielding **26** as an oil (101 mg, 22%). $C_{31}H_{30}N_2O_2$ (462.59); $^1$H NMR (400 MHz, $CDCl_3$): δ 1.18 – 1.31 (m, 4H), 1.52 – 1.69 (m, 4H), 2.42 (s, 3H), 3.61 (t, *J* = 7.2 Hz, 2H), 3.86-3.95 (m, 2H), 7.17 – 7.23 (m, 1H), 7.27 – 7.33 (m, 1H), 7.34 – 7.45 (m, 8H), 7.68 – 7.74 (m, 2H), 7.80 – 7.86 (m, 2H); MS (EI): *m/z* (%) 462 (82) [$M^+$], 246 (100).

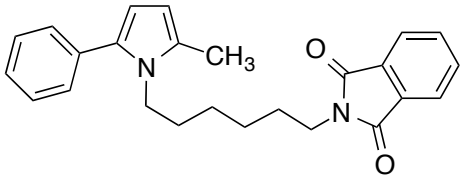
**Phenyl *N*-[6-(2-methyl-3,5-diphenylpyrrol-1-yl)hexyl]carbamate (27)**



To a solution of **26** (88 mg, 0.19 mmol) in ethanol (5 mL) was added aqueous hydrazine-hydrate solution (24%) (236 µL, 4.76 mmol) and the mixture was heated under reflux for 1 h. After cooling, $CHCl_3$ and 10% aqueous KOH were added. Then the mixture was diluted with water and the pH adjusted to about 10 with 1 M aqueous HCl. The organic phase was separated and aqueous phase extracted exhaustively with $CHCl_3$. The combined organic phases were dried over anhydrous sodium sulfate and concentrated. The residue was dissolved in dry $CH_2Cl_2$ (5 mL) and treated under nitrogen at 0 °C with triethylamine (25 µL, 0.18 mmol) and phenyl chloroformate (25 µL, 0.20 mmol). The mixture was stirred at 0 °C for 30 min and at room temperature for an additional 30 min. The reaction mixture was poured into half-saturated brine and extracted exhaustively with $CH_2Cl_2$. The organic layers were combined, dried over anhydrous sodium sulfate,

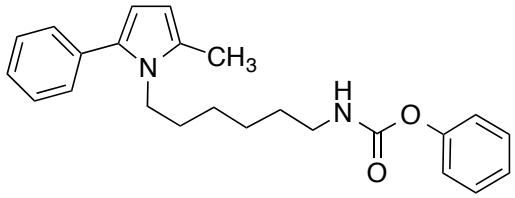
and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1 to 8:2) to afford **27** as a solid (68 mg, 79%). $C_{30}H_{32}N_2O_2$ (452.60); mp 114 – 116 °C, $^1$H NMR (400 MHz, $CDCl_3$): δ 1.16 – 1.32 (m, 4H), 1.41 – 1.52 (m, 2H), 1.59 – 1.68 (m, 2H), 2.44 (s, 3H), 3.13 – 3.30 (m, 2H), 3.89 – 3.98 (m, 2H), 4.52 – 5.06 (m, 1H), 6.30 (s, 1H), 7.09 – 7.15 (m, 2H), 7.16 – 7.24 (m, 2H), 7.30 – 7.47 ppm (m, 11H), $^1$H NMR (400 MHz, [$D_6$]DMSO): δ 1.09 – 1.26 (m, 4H), 1.29-1.45 (m, 2H), 1.47 – 1.61 (m, 2H), 2.38 (s, 3H), 2.92 – 3.10 (m, 2H), 3.87 – 3.97 (m, 2H), 6.24 (s, 1H), 7.04 – 7.11 (m, 2H), 7.15 – 7.22 (m, 2H), 7.29 – 7.47 ppm (m, 11H), 7.69 (t, *J* = 5.7 Hz, 1H), $^{13}$C NMR (101 MHz, [$D_6$]DMSO): δ 11.2, 25.6, 25.7, 29.0, 30.4, 40.3, 43.6, 108.0, 121.2, 121.7, 124.8, 125.0, 125.9, 126.8, 127.5, 128.4, 128.5, 128.6, 129.2, 132.9, 133.6, 137.0, 151.1, 154.3; HRMS-ESI $[M+H]^+$ calculated: 453.2537, found: 453.2553.

**2-[6-(2-Methyl-5-phenylpyrrol-1-yl)hexyl]isoindolin-1,3-dione (29)**



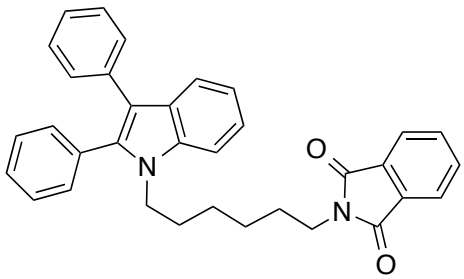

To a solution of 2-methyl-5-phenylpyrrol (**28**) (200 mg, 1.27 mmol) in dry DMSO (20 mL) was added under nitrogen K-*tert*-butylate (157 mg, 1.40 mmol). The mixture was stirred at 70 °C for 15 min. Then a solution of *N*-(6-bromohexyl)phthalimide (493 mg, 1.59 mmol) in dry DMSO was added dropwise over 2 min and the solution was stirred at 70 °C for an additional 6 h. The reaction mixture was cooled, poured into brine and extracted exhaustively with ethyl acetate. The organic extracts were combined and washed three times with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 19:1 to 9:1) yielding **29** as an oil (171 mg, 35%). $C_{25}H_{26}N_2O_2$ (386.5); $^1$H NMR (400 MHz, $CDCl_3$): δ 1.12 – 1.24 (m, 4H), 1.48 – 1.61 (m, 4H), 2.29 (s, 3H), 3.59 (t, *J* = 7.3 Hz, 2H), 3.82 – 3.88 (m, 2H), 5.92 (d, *J* = 3.4 Hz, 1H), 6.06 (d, *J* = 3.4 Hz, 1H), 7.23 – 7.29 (m, 1H), 7.31 – 7.40 (m, 4H), 7.68 – 7.74 (m, 2H), 7.80 – 7.87 ppm (m, 2H); MS (EI) *m/z* (%): 386 (100) $M^+$, 170 (91).

**Phenyl *N*-[6-(2-methyl-5-phenylpyrrol-1-yl)hexyl]carbamate (30)**



To a solution of **29** (145 mg, 0.38 mmol) in ethanol (30 mL) was added aqueous hydrazine-hydrate solution (24%) (469 mg, 2.25 mmol) and the mixture was heated under reflux for 2 h. The solvent was concentrated and the residue treated with brine (30 mL). After adjusting the pH to about 9 using 1M NaOH and 1M HCl, the mixture was exhaustively extracted with $CHCl_3$. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. The residue consisting of 6-(2-methyl-5-phenylpyrrol-1-yl)hexan-1-amine (85 mg, 0.33 mmol) was dissolved in dry $CH_2Cl_2$ (20 mL) and treated under a nitrogen atmosphere at 0 °C with triethylamine (34 mg, 0.33 mmol) and phenyl chloroformate (56 mg, 0.35 mmol). The mixture was stirred at 0 °C for 30 min and at room temperature for another 30 min. Then the reaction mixture was poured into half-saturated brine (20 mL) and extracted exhaustively with $CH_2Cl_2$. The organic extracts were combined, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 9:1 to 8:2) yielding **30** as an solid (104 mg, 73%). $C_{24}H_{28}N_2O_2$ (376.5); mp 59 – 60 °C; $^1$H NMR (400 MHz, $CDCl_3$): δ 1.10 – 1.28 (m, 4H), 1.37 – 1.49 (m, 2H), 1.50 – 1.61 (m, 2H), 2.32 (s, 3H), 3.08 – 3.30 (m, 2H), 3.80 – 3.98 (m, 2H), 4.54 – 5.06 (m, 1H), 5.96 (d, $J$ = 3.2 Hz, 1H), 6.09 (d, $J$ = 3.4 Hz, 1H), 7.12 (d, $J$ = 7.7 Hz, 2H), 7.19 (t, $J$ = 7.4 Hz, 1H), 7.27 – 7.44 ppm (m, 7H); $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ 12.5, 25.6, 29.0, 30.4, 40.2, 43.3, 106.7, 107.6, 121.7, 124.8, 126.4, 128.2, 128.5, 129.2, 129.4, 132.9, 134.1, 151.1, 154.2 ppm; HRMS-APCI $[M+H]^+$ calculated: 377.2224, found: 377.2224.

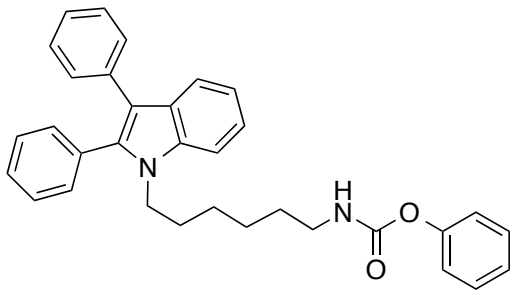
**2-[6-(2,3-Diphenylindol-1-yl)hexyl]isoindoline-1,3-dione (31)**



The compound was prepared from 2,3-diphenylindole (150 mg, 0.56 mmol) according to the procedure described for the preparation of **26**. The crude product was purified by

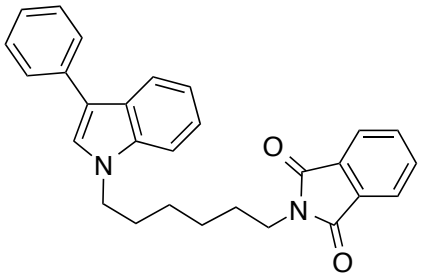
silica gel chromatography (hexanes/ethyl acetate, 9:1 to 8:2) yielding **31** as an oil (105 mg, 38%). $C_{34}H_{30}N_2O_2$ (498.63); $^1$H NMR (400 MHz, $CDCl_3$): δ 1.14 – 1.23 (m, 4H), 1.48 – 1.58 (m, 2H), 1.62 – 1.73 (m, 2H), 3.55 – 3.61 (m, 2H), 4.04 – 4.11 (m, 2H), 7.13 – 7.19 (m, 2H), 7.21 – 7.43 (m, 11H), 7.67 – 7.74 (m, 2H), 7.79 (d, *J* = 7.9 Hz, 1H), 7.80 – 7.87 (m, 2H); MS (EI): *m/z* (%) 498 (100) [$M^+$], 282 (51).

**Phenyl *N*-[6-(2,3-diphenylindol-1-yl)hexyl]carbamate (32)**




Compound **32** was obtained from **31** (80 mg, 0.16 mmol) in a similar manner as described for the synthesis of **27**. Yield: 33 mg, 42%. $C_{33}H_{32}N_2O_2$ (488.63); mp 44 – 46 °C, $^1$H NMR (400 MHz, $CDCl_3$): δ 1.15 – 1.24 (m, 4H), 1.37 – 1.49 (m, 2H), 1.64 – 1.74 (m, 2H), 3.10 – 3.27 (m, 2H), 4.08 – 4.15 (m, 2H), 4.58 – 4.98 (m, 1H), 7.07 – 7.46 (m, 19H), 7.81 (d, *J* = 7.9 Hz, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$): δ 26.1, 26.3, 29.6, 29.7, 41.0, 43.5, 109.9, 115.3, 119.7, 120.1, 121.5, 122.0, 125.2, 125.4, 127.2, 128.1, 128.4, 129.2, 129.8, 131.1, 132.2, 135.1, 136.3, 137.4, 151.0, 154.5; HRMS-ESI [*M*+H]$^+$ calculated: 489.2537, found: 489.2528.

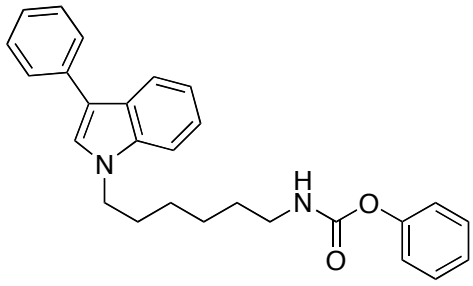
**2-[6-(3-Phenylindol-1-yl)hexyl]isoindolin-1,3-dione (33)**




To a solution of 3-phenylindole (250 mg, 0.59 mmol) in dry DMSO (20 mL) was added under nitrogen sodium hydride (60% in mineral oil) (62 mg, 1.55 mmol). The mixture was stirred at 110 °C for 5 min. Then a solution of *N*-(6-bromohexyl)phthalimide (314 mg, 1.0 mmol) in dry DMSO (2 mL) was added and the solution was stirred at 110 °C for an additional 3 h. The reaction mixture was poured into brine (30 mL) and extracted exhaustively with ethyl acetate. The combined organic extracts were washed twice with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 19:1)

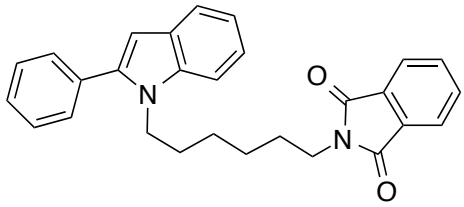
yielding **33** as an oil (158 mg, 63%). $C_{28}H_{26}N_2O_2$ (422.5); $^1$H NMR (400 MHz, [D$_6$]DMSO): δ 1.26 – 1.34 (m, 4H), 4.19 (t, *J* = 7.1 Hz), 7.07-7.13 (m, 1H), 7.14 – 7.25 (m, 2H), 7.39 – 7.45 (m, 2H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.63 – 7.68 (m, 2H), 7.71 (s, 1H), 7.80 – 7.88 ppm (m, 5H); HRMS-APCI $[M+H]^+$ calculated: 423.2067, found: 423.2036.

**Phenyl *N*-[6-(3-phenylindol-1-yl)hexyl]carbamate (34)**



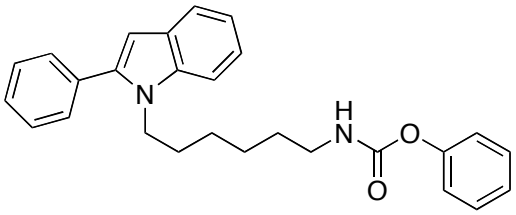

To a solution of **33** (144 mg, 0.34 mmol) in ethanol (30 mL) was added aqueous hydrazine-hydrate solution (24%) (569 mg, 2.73 mmol) and the mixture was heated under reflux for 3 h. The solvent was concentrated and the residue treated with brine (30 mL). After adjusting the pH to about 9 using 1M NaOH and 1M HCl, the mixture was exhaustively extracted with $CHCl_3$. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. The residue consisting of 6-(3-phenylindole-1-yl)hexan-1-amine (94 mg, 0.32 mmol) was dissolved in dry THF (20 mL) and successively treated with ethyl(diisopropyl)amine (42 mg, 0.83 mmol) and phenyl chloroformate (51 mg, 0.83 mmol). After stirring the mixture at room temperature for 2 h, silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and eluted with hexane/ethyl acetate (19:1) to afford **34** as an oil (110 mg, 78%). $C_{27}H_{28}N_2O_2$ (412.5); $^1$H NMR (400 MHz, [D$_6$]DMSO): δ 1.25 – 1.40 (m, 4H), 1.40 – 1.51 (m, 2H), 1.76 – 1.86 (m, 2H), 2.99-3.17 (m, 2H), 4.21 (t, *J* = 7.1 Hz, 2H), 7.05 – 7.26 (m, 6H), 7.32 – 7.39 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.67 (dd, *J* = 8.1 Hz und 1.0 Hz, 2H), 7.70 – 7.75 (m, 2H), 7.88 ppm (d, *J* = 7.9 Hz, 1H); $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ 25.9, 26.0, 29.1, 29.7, 40.4, 45.5, 110.3, 114.9, 119.3, 119.7, 121.5, 121.7, 124.8, 125.3, 125.3, 126.5, 126.7, 128.8, 129.2, 135.5, 136.6, 151.1, 154.3 ppm (N*C*OO); HRMS-APCI $[M+H]^+$ calculated: 413.2224, found: 413.2229.

**2-[6-(2-Phenylindol-1-yl)hexyl]isoindolin-1,3-dione (35)**



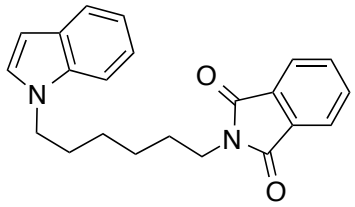
To a solution of 2-phenylindole (200 mg, 1.0 mmol) in dry DMF (30 mL) was added under nitrogen sodium hydride (60% in mineral oil) (40 mg, 1.0 mmol). The mixture was stirred at 60 °C for 30 min. Then *N*-(6-bromohexyl)phthalimide (314 mg, 1.0 mmol) was added and the solution was stirred at 60 °C for an additional 2 h. The reaction mixture was poured into half-saturated brine (30 mL) and extracted exhaustively with $CH_2Cl_2$. The organic extracts were combined, washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 19:1) yielding **35** as an oil (66 mg, 16%). $C_{28}H_{26}N_2O_2$ (422.5); $^1$H NMR (400 MHz, $CDCl_3$): δ 1.13 – 1.23 (m, 4H), 1.48 – 1.61 (m, 2H), 1.62 – 1.75 (m, 2H), 3.55 – 3.61 (m, 2H), 4.11 – 4.17 (m, 2H), 6.50 (s, 1H), 7.09 – 7.14 (m, 1H), 7.18 – 7.24 (m, 1H), 7.34 – 7.41 (m, 2H), 7.43 – 7.50 (m, 4H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.67 – 7.73 (m, 2H), 7.80 – 7.86 ppm (m, 2H); MS (EI) *m/z* (%): 422 (100) $M^+$, 206 (52).

**Phenyl *N*-[6-(2-phenylindol-1-yl)hexyl]carbamate (36)**



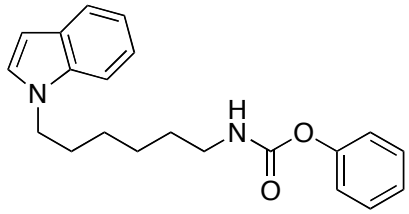
Compound **36** was prepared from **35** (47 mg, 0.16 mmol) using a similar procedure as described for the synthesis of **30**. After addition of phenyl chloroformate, the mixture was stirred at room temperature overnight. Chromatography on silica gel (hexane/ethyl acetate/$CH_2Cl_2$, 8:1:1) yielded **36** as an oil (26 mg, 40%). $C_{27}H_{28}N_2O_2$ (412.5); $^1$H NMR (400 MHz, [$D_6$]DMSO): δ 1.00 – 1.18 (m, 4H), 1.25 – 1.36 (m, 2H), 1.50 – 1.61 (m, 2H), 2.88 – 3.06 (m, 2H), 4.20 (t, *J* = 7.4 Hz, 2H), 6.51 (s, 1H), 7.03 – 7.09 (m, 3H), 7.13 – 7.21 (m, 2H), 7.32 – 7.39 (m, 2H), 7.41 – 7.47 (m, 1H), 7.48 – 7.58 (m, 6H), 7.65 ppm (t, *J* = 5.6 Hz, 1H); $^{13}$C NMR (101 MHz, [$D_6$]DMSO): δ 25.6, 25.7, 29.0, 29.3, 40.3, 43.1, 101.7, 110.5, 119.5, 120.2, 121.4, 121.8, 124.8, 127.6, 128.0, 128.7, 129.0, 129.2, 132.7, 137.2, 140.8, 151.1, 154.3 ppm; HRMS-ESI [*M*+Na]$^+$ calculated 435.2043, found 435.2051.

**2-(6-Indol-1-ylhexyl)isoindolin-1,3-dione (37)**



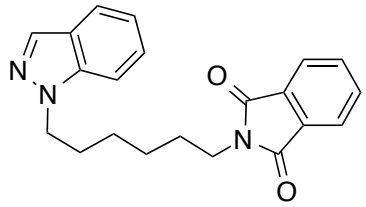
To a solution of indole (150 mg, 1.28 mmol) in dry DMSO (10 mL) was added under nitrogen sodium hydride (60% in mineral oil) (61 mg, 1.5 mmol). The mixture was stirred at 110 °C for 10 min. Then a solution of *N*-(6-bromohexyl)phthalimide (397 mg, 1.28 mmol) and a catalytical amount of potassium iodide in dry DMSO (20 mL) was added and the solution was stirred at 110 °C for an additional 4 h. The reaction mixture was poured into brine (30 mL) and extracted exhaustively with $CH_2Cl_2$. The combined organic extracts were washed three times with brine, dried over anhydrous sodium sulfate, and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 9:1) yielding **37** as an oil (152 mg, 34%). $C_{22}H_{22}N_2O_2$ (346.4); $^1$H NMR (300 MHz, $CDCl_3$): δ 1.29 – 1.43 (m, 4H), 1.54 – 1.72 (m, 2H), 1.76 – 1.91 (m, 2H), 3.61 – 3.70 (m, 2H), 4.05v4.15 (m, 2H), 6.47 (dd, *J* = 3.1 Hz und 0.7 Hz, 1H), 7.05 – 7.11 (m, 2H), 7.15 – 7.22 (m, 1H), 7.29 – 7.35 (m, 1H), 7.59 – 7.63 (m, 1H), 7.67 – 7.74 (m, 2H), 7.80 – 7.87 ppm (m, 2H); MS (EI) *m/z* (%): 346 (37) $M^+$, 130 (100).

**Phenyl *N*-(6-indol-1-ylhexyl)carbamate (38)**



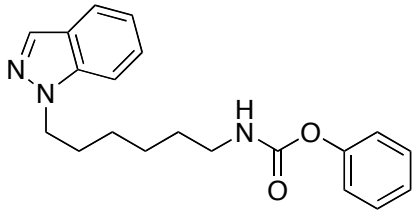
Compound **38** was prepared from **37** (100 mg, 0.29 mmol) using a similar procedure as described for the synthesis of **34**. Chromatography on silica gel (hexane/ethyl acetate, 9:1) yielded **38** as an oil (70 mg, 74%). $C_{21}H_{24}N_2O_2$ (336.4); mp 66-67°C; $^1$H NMR (400 MHz, [$D_6$]DMSO): δ 1.20 – 1.38 (m, 4H), 1.37 – 1.50 (m, 2H), 1.69 – 1.81 (m, 2H), 2.97 – 3.16 (m, 2H), 4.15 (t, *J* = 7.0 Hz, 2H), 6.41 (d, *J* = 3.0 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 7.04 – 7.15 (m, 3H), 7.18 (t, *J* = 7.3 Hz, 1H), 7.31 – 7.40 (m, 3H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.54 (d, *J* = 7.8 Hz, 1H), 7.71 ppm (t, *J* = 5.5 Hz, 1H); HRMS-ESI [*M*+Na]$^+$ calculated: 359.1730, found: 359.1728.

**2-(6-Indazol-1-ylhexyl)isoindolin-1,3-dione (39)**



To a solution of indazole (150 mg, 1.27 mmol) in dry DMSO (20 mL) was added $K_2CO_3$ (351 mg, 2.53 mmol) und *N*-(6-bromohexyl)phthalimide (394 mg, 1.27 mmol). The mixture was heated at 50 °C for 5 h, poured into water and extracted exhaustively with $CH_2Cl_2$. The combined organic layers were washed twice with water, dried over anhydrous sodium sulfate and concentrated. The crude material was purified by silica gel chromatography (hexane/ethyl acetate, 8:2 to 1:1) yielding **39** as an oil (96 mg, 22%). $C_{21}H_{21}N_3O_2$ (347.4); mp 80 – 81°C; $^1$H NMR (400 MHz, $CDCl_3$): δ 1.34 – 1.46 (m, 4H), 1.62 – 1.72 (m, 2H), 1.97 – 2.08 (m, 2H), 3.66 (t, *J* = 7.2 Hz, 2H), 4.43 (t, *J* = 7.1 Hz, 2H), 7.06-7.12 (m, 1H), 7.27 - 7.32 (m, 1H), 7.65 (d, *J* = 8.4 Hz, 1H), 7.68 – 7.73 (m, 3H), 7.80 – 7.86 (m, 2H), 7.92 ppm (s, 1H); MS (EI) *m/z* (%): 347 (98) $M^+$, 131 (100).

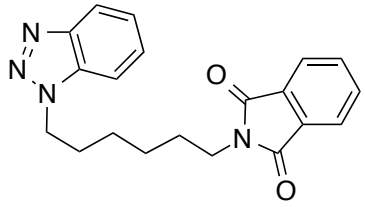
**Phenyl *N*-(6-indazol-1-ylhexyl)carbamate (40)**



To a solution of **39** (96 mg, 0.28 mmol) in ethanol (30 mL) was added aqueous hydrazine-hydrate solution (24%) (346 mg, 1.66 mmol) and the mixture was heated under reflux for 2 h. The solvent was concentrated and the residue treated with brine (30 mL). After adjusting the pH to about 9 using 1M NaOH and 1M HCl, the mixture was exhaustively extracted with $CH_2Cl_2$. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. The residue consisting of 6-(indazol-1-yl)hexan-1-amine (60 mg, 0.28 mmol) was dissolved in dry THF (15 mL) and successively treated with triethylamine (28 mg, 0.28 mmol) and phenyl chloroformate (44 mg, 0.28 mmol). After stirring the mixture at room temperature for 2 h, the mixture was poured into water and extracted exhaustively with $CH_2Cl_2$. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. Chromatography on silica gel (hexane/ethyl acetate, 9:1 to 6:4) yielded **40** as a wax-like substance (51 mg, 54%); $C_{20}H_{23}N_3O_2$ (337.4); $^1$H NMR (400 MHz, [$D_6$]DMSO): δ 1.29 – 1.38 (m, 2H), 1.39 – 1.48 (m, 2H), 1.87 – 1.97 (m, 2H), 2.97 – 3.11 (m, 2H), 4.41 (t, *J* = 7.0 Hz, 2H), 6.99 –

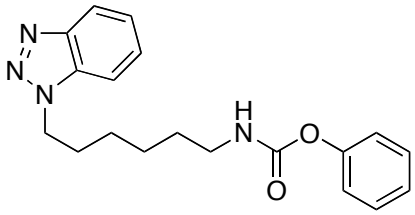
7.04 (m, 1H), 7.07 (d, $J$ = 7.7 Hz, 2H), 7.15 – 7.24 (m, 2H), 7.36 (t, $J$ = 7.8 Hz, 2H), 7.59 (d, $J$ = 8.7 Hz, 1H), 7.65 – 7.75 (m, 2H), 8.34 ppm (s, 1H); $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ 25.7, 25.7, 29.1, 30.0, 40.4, 52.6, 116.9, 120.5, 120.8, 121.3, 121.8, 123.6, 124.8, 125.2, 129.2, 147.9, 151.1, 154.3 ppm; HRMS-ESI [$M$+H]$^+$ calculated: 338.1863, found: 338.1865.

**2-(6-Benzotriazol-1-ylhexyl)isoindolin-1,3-dione (41)**



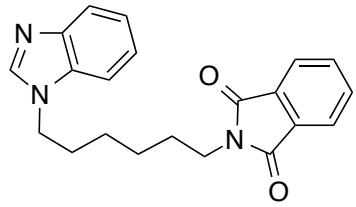
Compound **41** was prepared from benzotriazole (150 mg, 1.26 mmol) using a similar procedure as described for the synthesis of **43**. Chromatography on silica gel (hexane/ethyl acetate, 7:3) yielded **41** as a solid (223 mg, 51%). $C_{20}H_{20}N_4O_2$ (348.4); mp 81 – 82°C; $^1$H NMR (300 MHz, $CDCl_3$): δ 1.31 – 1.48 (m, 4H), 1.59 – 1.74 (m, 2H), 1.94 – 2.09 (m, 2H), 3.62 – 3.70 (m, 2H), 4.63 (t, $J$ = 7.1 Hz, 2H), 7.32 – 7.39 (m, 1H), 7.44 – 7.54 (m, 2H), 7.67 – 7.74 (m, 2H), 7.79 – 7.87 (m, 2H), 8.05 ppm (dt, $J$ = 8.3, 1.0 Hz, 1H); MS (ESI) [$M$+Na]$^+$: 371.

**Phenyl *N*-(6-benzotriazol-1-ylhexyl)carbamate (42)**



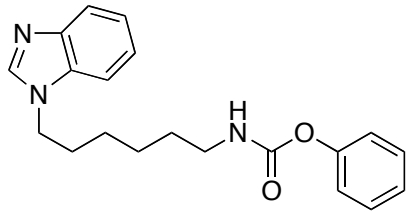
Compound **42** was prepared from **41** (100 mg, 0.29 mmol) using a similar procedure as described for the synthesis of **34**. Chromatography on silica gel (hexane/ethyl acetate, 7:3) yielded **42** as a solid (97 mg, 99%). $C_{19}H_{22}N_4O_2$ (338.4); mp 82 – 83°C; $^1$H NMR (400 MHz, [D$_6$]DMSO): δ 1.21 – 1.37 (m, 4H), 1.38 – 1.48 (m, 2H), 1.86 – 1.97 (m, 2H), 2.95 – 3.15 (m, 2H), 4.71 (t, $J$ = 7.0 Hz, 2H), 7.07 (d, $J$ = 7.8 Hz, 2H), 7.18 (t, $J$ = 7.4 Hz, 1H), 7.31 – 7.43 (m, 3H), 7.54 (t, $J$ = 7.6 Hz, 1H), 7.71 (t, $J$ = 5.6 Hz, 1H), 7.89 (d, $J$ = 8.3 Hz, 1H), 8.04 ppm (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (101 MHz, [D$_6$]DMSO): δ 25.6, 25.7, 29.0, 29.2, 40.3, 47.4, 110.6, 119.1, 121.8, 123.9, 124.8, 127.1, 129.2, 132.8, 145.1, 151.1, 154.3 ppm; HRMS-ESI [$M$+Na]$^+$ calculated: 361.1635, found: 361.1632.

**2-(6-Benzimidazol-1-ylhexyl)isoindolin-1,3-dione (43)**



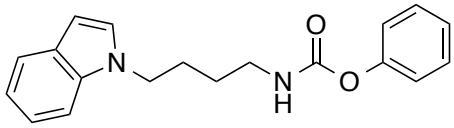
To a solution of benzimidazole (200 mg, 1.69 mmol) in dry acetonitrile (20 mL) was added $K_2CO_3$ (281 mg, 2.03 mmol), *N*-(6-bromohexyl)phthalimide (525 mg, 1.69 mmol) und a catalytical amount of potassium iodide. After heating under reflux for 2 h, the mixture was cooled, poured into brine (20 mL) and extracted exhaustively with $CH_2Cl_2$. The combined organic layers were dried over anhydrous sodium sulfate and concentrated. Chromatography on silica gel (hexane/ethyl acetate, 7:3) yielded **43** as an oil (73 mg, 12%); $C_{21}H_{21}N_3O_2$ (347.4); $^1$H NMR (300 MHz, $CDCl_3$): δ1.34 – 1.44 (m, 4H), 1.61 – 1.73 (m, 2H), 1.82 – 1.95 (m, 2H), 3.67 (t, *J* = 7.1 Hz, 2H), 4.17 (t, *J* = 7.1 Hz, 2H), 7.26 – 7.34 (m, 2H), 7.36 – 7.43 (m, 1H), 7.67 – 7.75 (m, 2H), 7.78 – 7.87 (m, 3H), 7.93 ppm (s, 1H); MS (EI) *m/z* (%): 347 (44) $M^+$, 131 (100).

**Phenyl *N*-(6-benzimidazol-1-ylhexyl)carbamate (44)**



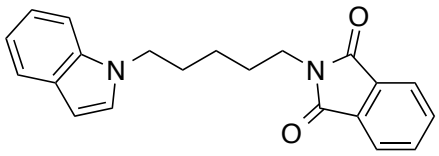
Compound **44** was prepared from **43** (69 mg, 0.20 mmol) using a similar procedure as described for the synthesis of **34**. After addition of phenyl chloroformate, the mixture was stirred at room temperature overnight. Chromatography on silica gel (hexane/THF, 3:7) yielded **44** as a solid (22 mg, 32%). $C_{20}H_{23}N_3O_2$ (337.4); mp 88 – 89°C; $^1$H NMR (400 MHz, [$D_6$]DMSO): δ 1.19-1.38 (m, 4H), 1.38 – 1.48 (m, 2H), 1.74 – 1.85 (m, 2H), 2.95 – 3.15 (m, 2H), 4.24 (t, *J* = 7.0 Hz, 2H), 7.07 (d, *J* = 7.7 Hz, 2H), 7.15-7.28 (m, 3H), 7.36 (t, *J* = 7.9 Hz, 2H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.72 (t, *J* = 5.6 Hz, 1H), 8.23 ppm (s, 1H); $^{13}$C NMR (101 MHz, [$D_6$]DMSO): δ 25.7, 25.8, 29.1, 29.3, 40.3, 44.0, 110.4, 119.4, 121.4, 121.8, 122.2, 124.8, 129.2, 133.8, 143.4, 144.0, 151.1, 154.3 ppm; HRMS-ESI [*M*+H]$^+$ calculated: 338.1863, found: 338.1855.

**Phenyl *N*-(4-indol-1-ylbutyl)carbamate (45)**



To sodium hydride (60% dispersion in mineral oil) (410 mg, 10.25 mmol) in dry DMSO (20 mL) was added indole (1.0 g, 8.54 mmol). After stirring the mixture at room temperature for 1 h, it was added dropwise to a solution of *N*-(4-bromobutyl)phthalimide (2.4 g, 8.54 mmol) in dry DMSO (20 mL) and heated at 100°C for 3 h. The cooled reaction mixture was poured into brine and extracted exhaustively with $CH_2Cl_2$. The combined organic extracts were dried over sodium sulfate, and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 4:1). The obtained crude 2-[4-(indol-1-yl)butyl]isoindoline-1,3-dione (1.39 g) was dissolved in ethanol (80 mL) and treated with aqueous hydrazine-hydrate solution (24%) (7.26 g, 34.8 mmol). The mixture was heated under reflux for 4 h. After distilling off most of the ethanol, brine was added and a pH of 10 was adjusted with dilute NaOH. The aqueous solution was extracted exhaustively with $CHCl_3$. The combined organic phases were dried over anhydrous sodium sulfate and concentrated. The residue consisting of 4-(indol-1-yl)butylamine (726 mg) was dissolved in dry THF (25 mL) and treated with ethyl(diisopropyl)amine (557 mg, 4.3 mmol). Then phenyl chloroformate (682 mg, 4.3 mmol) was added dropwise and the mixture was stirred at room temperature overnight. Silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and eluted with hexane/ethyl acetate (9:1 to 8:2) to afford **45** as a solid (561 mg, 21%). $C_{19}H_{20}N_2O_2$ (308.38); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.50 – 1.59 (m, 2H), 1.86 – 1.94 (m, 2H), 3.21 – 3.27 (m, 2H), 4.17 (t, $J$ = 6.9 Hz, 2H), 5.07 (t, $J$ = 6.0 Hz, 1H), 6.53 (d, $J$ = 3.1 Hz, 1H), 7.09 – 7.17 (m, 4H), 7.19 – 7.25 (m, 2H), 7.34 – 7.41 (m, 3H), 7.66 – 7.69 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 27.42, 40.69, 45.90, 101.26, 109.42, 119.40, 121.11, 121.56, 121.67, 125.40, 127.85, 128.68, 129.38, 135.97, 151.05, 154.82; HRMS-APCI [$M$+$H^+$] calculated: 309.1598, found: 309.1605.

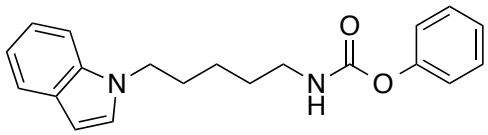
**2-[5-(Indol-1-yl)pentyl]isoindoline-1,3-dione (46)**



To sodium hydride (60% dispersion in mineral oil) (243 mg, 6.08 mmol) in dry DMSO (10 mL) was added indole (593 mg, 5.06 mmol). After stirring the mixture at room temperature for 30 min, it was added dropwise to a solution of *N*-(5-bromopentyl)-phthalimide (1.5 g, 5.06 mmol) in dry DMSO (10 mL) and heated at 110°C for 4 h. The

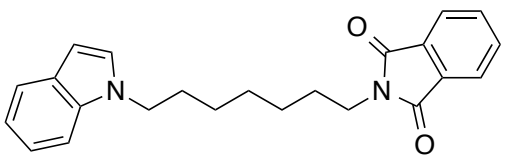
cooled reaction mixture was poured into brine and extracted exhaustively with $CH_2Cl_2$. The combined organic extracts were dried over anhydrous sodium sulfate and concentrated. The residue was chromatographed on silica gel (hexane/ethyl acetate, 9:1) to yield **46** as an oil (452 mg, 27%). $C_{21}H_{20}N_2O_2$ (332.40); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.34 – 1.43 (m, 2H), 1.67 – 1.76 (m, 2H), 1.85 – 1.94 (m, 2H), 3.67 (t, *J* = 7.2 Hz, 2H), 4.12 (t, *J* = 7.2 Hz, 2H), 6.46 (d, *J* = 3.1 Hz, 1H), 7.05 – 7.11 (m, 2H), 7.15 – 7.22 (m, 1H), 7.33 (d, *J* = 8.2 Hz, 1H), 7.61 (d, 1H), 7.69 – 7.73 (m, 2H), 7.82 – 7.86 (m, 2H); MS (EI): *m/z* (%) 332.2 (100) [$M^+$].

**Phenyl *N*-[5-(indol-1-yl)pentyl]carbamate (47)**



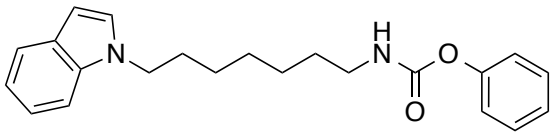

Compound **46** (445 mg, 1.34 mmol) was dissolved in ethanol (30 mL) and treated with aqueous hydrazine-hydrate solution (24%) (2.21 mL, 10.7 mmol). The mixture was heated under reflux for 3 h. After distilling off most of the ethanol, brine was added and a pH of 10 was adjusted with dilute NaOH. The aqueous solution was extracted exhaustively with $CHCl_3$. The combined organic phases were dried over anhydrous sodium sulfate and concentrated. The residue consisting of 5-(indol-1-yl)pentan-1-amine (215 mg) was dissolved in dry THF (20 mL) and treated with ethyl(diisopropyl)amine (151 mg, 1.17 mmol). Then phenyl chloroformate (185 mg, 1.18 mmol) was added dropwise and the mixture was stirred at room temperature overnight. Silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and eluted with hexane/ethyl acetate (9:1 to 8:2) to afford **47** as an oil (376 mg, 87%). $C_{20}H_{22}N_2O_2$ (322.41); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.33 – 1.42 (m, 2H), 1.54 – 1.62 (m, 2H), 1.84 – 1.94 (m, 2H), 3.19 – 3.26 (m, 2H), 4.14 (t, *J* = 7.0 Hz, 2H), 4.95 – 5.03 (m, 1H), 6.51 (d, *J* = 3.2 Hz, 1H), 7.08 – 7.14 (m, 4H), 7.17 – 7.25 (m, 2H), 7.33 – 7.40 (m, 3H), 7.62 – 7.68 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 24.23, 29.62, 29.97, 41.09, 46.30, 101.16, 109.44, 119.37, 121.12, 121.53, 121.70, 125.39, 127.89, 128.70, 129.40, 136.02, 151.13, 154.74; HRMS-APCI [*M*+$H^+$] calculated: 323.1754, found: 323.1802.

**2-[7-(Indol-1-yl)heptyl]isoindoline-1,3-dione (48)**



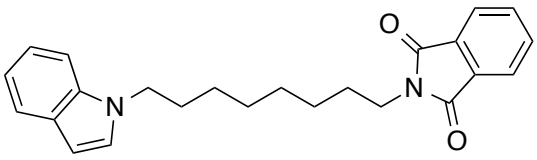
Compound **48** was prepared from indole (535 mg, 4.57 mmol) and *N*-(7-bromoheptyl)phthalimide (1.48 g, 4.57 mmol) using a similar procedure as described for the synthesis of **46**. Yield: 391 mg, 24%. An aliquot of the product was dissolved in a small amount of DMSO and was cleaned up by reversed phase HPLC on a preparative Knauer RP18 Eurospher II 5 µm column (20 mm inside diameter x 250 mm) with a Knauer RP18 Eurospher II 5 µm guard column (20 mm inside diameter x 30 mm). Elution was performed with acetonitrile/water (80/20, v/v) at a flow rate of 25 mL/min and the effluent was monitored at 254 nm. Pure compound **48** was obtained as solid after distilling off the organic solvent and freeze-drying the remaining aqueous phase. $C_{23}H_{24}N_2O_2$ (360.46); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.28 – 1.41 (m, 6H), 1.60 – 1.70 (m, 2H), 1.77 – 1.87 (m, 2H), 3.66 (t, *J* = 7.2 Hz, 2H), 4.10 (t, *J* = 7.1 Hz, 2H), 6.47 (d, 1H), 7.05 – 7.12 (m, 2H), 7.15 – 7.22 (m, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.66 – 7.74 (m, 2H), 7.78 – 7.87 (m, 2H); MS (EI): *m/z* (%) 360.3 (83) [$M^+$], 130.1 (100).

**Phenyl *N*-[7-(indol-1-yl)heptyl]carbamate (49)**




Compound **49** was prepared from **48** (385, 1.07 mmol) using a similar procedure as described for the synthesis of **47**. Yield: 225 mg, 60%; $C_{22}H_{26}N_2O_2$ (350.46); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.30 – 1.37 (m, 6H), 1.47 – 1.58 (m, 2H), 1.79 – 1.90 (m, 2H), 3.18 – 3.27 (m, 2H), 4.12 (t, *J* = 7.1 Hz, 2H), 4.94 – 5.04 (m, 1H), 6.50 (d, *J* = 3.1 Hz, 1H), 7.08 – 7.15 (m, 4H), 7.16 – 7.24 (m, 2H), 7.31 – 7.39 (m, 3H), 7.62 – 7.67 (m, 1H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 26.68, 27.01, 28.98, 29.84, 30.26, 41.25, 46.47, 101.00, 109.49, 119.29, 121.07, 121.44, 121.72, 125.35, 127.93, 128.68, 129.39, 136.04, 151.18, 154.73; HRMS-APCI [*M*+$H^+$] calculated: 351.2067, found: 351.2099.

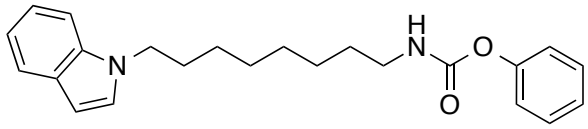
**2-[8-(Indol-1-yl)octyl]isoindoline-1,3-dione (50)**




Compound **50** was prepared from indole (347 mg, 2.96 mmol) and *N*-(8-bromooctyl)phthalimide (1.0 g, 2.96 mmol) using a similar procedure as described for the synthesis of **46**. Yield: 744 mg, 67%; $C_{24}H_{26}N_2O_2$ (374.48); $^1$H NMR (600 MHz, $CDCl_3$) δ 1.28 – 1.35 (m, 8H), 1.62 – 1.69 (m, 2H), 1.79 – 1.85 (m, 2H), 3.66 (t, *J* = 7.3 Hz, 2H), 4.10 (t, *J* = 7.2 Hz, 2H), 6.47 (d, *J* = 3.1 Hz, 1H), 7.08 – 7.10 (m, 2H), 7.18 –

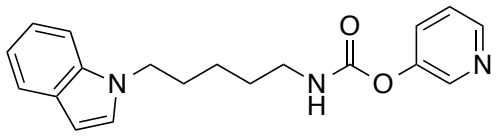
7.21 (m, 1H), 7.33 (d, $J$ = 8.2 Hz, 1H), 7.62 (d, $J$ = 7.9 Hz, 1H), 7.69 – 7.72 (m, 2H), 7.82 – 7.85 (m, 2H); MS (EI): *m/z* (%) 374.17 (17) [$M^+$], 130.02 (100).

**Phenyl *N*-[8-(indol-1-yl)octyl]carbamate (51)**



Compound **51** was prepared from **50** (711 mg, 1.90 mmol) using a similar procedure as described for the synthesis of **47**. Yield: 504 mg, 73%; $C_{23}H_{28}N_2O_2$ (364.49); $^1$H NMR (600 MHz, $CDCl_3$) δ 1.30 – 1.36 (m, 8H), 1.51 – 1.57 (m, 2H), 1.82 – 1.88 (m, 2H), 3.22 – 3.26 (m, 2H), 4.12 (t, $J$ = 7.1 Hz, 2H), 4.96 – 5.04 (m, 1H), 6.50 (d, $J$ = 3.1 Hz, 1H), 7.10 – 7.12 (m, 2H), 7.12 – 7.15 (m, 2H), 7.18 – 7.24 (m, 2H), 7.34 – 7.39 (m, 3H), 7.64 – 7.66 (m, 1H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 26.71, 27.02, 29.19, 29.23, 29.88, 30.31, 41.32, 46.48, 100.98, 109.49, 119.27, 121.05, 121.41, 121.70, 125.32, 127.89, 128.69, 129.37, 136.07, 151.21, 154.71; HRMS-APCI [$M$+$H^+$] calculated: 365.2224, found: 365.2266.

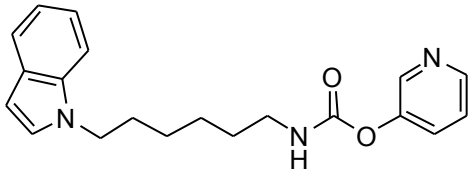
**Pyridin-3-yl *N*-[5-(indol-1-yl)pentyl]carbamate (52)**



Under a nitrogen atmosphere, pyridin-3-ol (371 mg, 3.90 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL). To this solution were slowly added at -30$^o$C trichloromethyl chloroformate (1.08 g, 5.46 mmol) and ethyl(diisopropyl)amine (504 mg, 3.90 mmol). The mixture was stirred at room temperature for 4 h. Then the solvent was distilled off to yield pyridin-3-yl carbonochloridate. An aliquot of this compound (373 mg, 2.37 mmol) was dissolved in dry THF (6 mL), and added dropwise to a solution of 5-(indol-1-yl)pentan-1-amine (479 mg, 2.37 mmol), which was obtained as described above in the synthesis of **47**, and ethyl(diisopropyl)amine (310 mg, 2.40 mmol) in dry THF (10 mL). After stirring at room temperature for 6 h, silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and eluted with hexane/ethyl acetate/$CH_2Cl_2$ (32:48:20) to afford **52** as an oil (66 mg). $C_{19}H_{21}N_3O_2$ (323.40); $^1$H NMR (600 MHz, $CDCl_3$) δ 1.33 – 1.39 (m, 2H), 1.54 – 1.60 (m, 2H), 1.85 – 1.91 (m, 2H), 3.20 – 3.24 (m, 2H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.10 – 5.16 (m, 1H), 6.49 (d, $J$ = 3.1 Hz, 1H), 7.07 – 7.11 (m, 2H), 7.18 – 7.21 (m, 1H), 7.27 – 7.31 (m, 1H), 7.32 – 7.35 (m, 1H), 7.48 – 7.52 (m, 1H), 7.61 – 7.64 (m, 1H), 8.40 – 8.46 (m, 2H); $^{13}$C NMR (151 MHz, $CDCl_3$) δ 24.22, 29.54, 29.95, 41.21, 46.27, 101.22, 109.42, 119.40,

121.14, 121.55, 123.88, 127.86, 128.72, 129.35, 136.04, 143.40, 146.26, 147.88, 153.90; HRMS-APCI [$M$+$H^+$] calculated: 324.1707, found: 324.1699.

**Pyridin-3-yl [6-(indol-1-yl)hexyl]carbamate (54)**




Under a nitrogen atmosphere, pyridin-3-ol (50 mg, 0.52 mmol) was dissolved in dry $CH_2Cl_2$ (8 mL). To this solution were slowly added at -30°C trichloromethyl chloroformate (144 mg, 0.73 mmol) and ethyl(diisopropyl)amine (67 mg, 0.52 mmol). The mixture was stirred at room temperature for 4 h. Then the solvent was distilled off, the residue dissolved in dry THF (5 mL), and the obtained solution added dropwise to a solution of 6-(indol-1-yl)hexan-1-amine (**53**) (124 mg, 0.57 mmol), prepared analogously to 5-(indol-1-yl)pentan-1-amine (see synthesis of compound **47**), and ethyl(diisopropyl)-amine (74 mg, 0.57 mmol) in dry THF (5 mL). After stirring at room temperature overnight, silica gel was added and the solvent was evaporated. The residue was transferred to the top of a silica gel column and eluted with hexane/ethyl acetate/$CH_2Cl_2$ (5:5:1) to afford **54** as an oil (12 mg). $C_{20}H_{23}N_3O_2$ (337,42); $^1$H NMR (400 MHz, $CDCl_3$) δ 1.33 – 1.40 (m, 4H), 1.50 – 1.60 (m, 2H), 1.82 – 1.91 (m, 2H), 3.19 – 3.27 (m, 2H), 4.13 (t, $J$ = 7.0 Hz, 2H), 5.06 – 5.15 (m, 1H), 6.49 (dd, $J$ = 3.1 Hz and 0.9 Hz, 1H), 7.08 – 7.13 (m, 2H), 7.21 (m, 1H), 7.28 – 7.36 (m, 2H), 7.50 – 7.55 (m, 1H), 7.62 – 7.65 (m, 1H), 8.42 – 8.46 (m, 2H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ 26.48, 26.77, 29.76, 30.24, 41.30, 46.41, 101.10, 109.45, 119.35, 121.12, 121.49, 123.90, 127.90, 128.70, 129.35, 136.04, 143.48, 146.33, 147.89, 153.92; HRMS-APCI [$M$+$H^+$] calculated: 338.1863, found: 338.1876.

## 2. Biological evaluation

### 2.1. FAAH inhibition assay

*Isolation of rat brain microsomes*

Two rat brains (female Sprague Dawley rats, Harlan-Winkelmann) were homogenized for 3 min under ice-cooling with a fivefold volume potassium phosphate buffer (0.1 M, pH 7.4 at 20 °C) containing EDTA (1 mM) using a Potter-Elvehjem homogenizer at 1000 – 1200 rpm. The homogenate was centrifuged at 1000 x g at 4 °C for 10 min and the resulting supernatant was centrifuged again at 10000 x g at 4 °C for 30 min. Finally, the supernatant was centrifuged at 40000 x g at 4 °C for 60 min. The obtained supernatant was discharged. The pellet was resuspended in 2 mL of ice-cold potassium phosphate buffer (0.1 M, pH 7.4) containing EDTA (1 mM) and stored in aliquots at -80 °C. Immediately before each incubation in dependence of the activity of FAAH in the preparation, an aliquot of about 50 µL was diluted with about 200 to 400 µL potassium phosphate buffer (0.1 M, pH 7.4) containing EDTA (1 mM) and homogenized shortly (2 x 5 s) at 0 °C with a Branson sonifier B15. Under the conditions applied, in the controls the peak area of the enzyme product 4-pyren-1-ylbutanoic acid amounted to about 70% of the peak area of the internal standard 6-pyren-1-ylhexanoic acid.

*Incubation procedure*

The substrate *N*-(2-hydroxyethyl)-4-pyren-1-ylbutanamide[1] was dissolved in methanol (2.5 mg/mL). An aliquot of this solution was thoroughly dried under a stream of nitrogen. The residue was resuspended by intense vortexing and sonication in a sonication bath in such an amount of a solution of 0.2% (m/v) Triton X-100 in phosphate buffered saline (0.01 M, pH 7.4 at 20 °C, prepared from tablets from Sigma-Aldrich, P4417) containing EDTA (1 mM), so that the concentration of the substrate was 114 µM. 88 µL of the obtained mixture were added to 2 µL of a DMSO solution of inhibitor or to 2 µL of DMSO in case of the controls. The mixture was pre-incubated for 10 min at 37 °C. Then the enzymatic reaction was started by adding 10 µL rat brain microsome preparation and continued at 37 °C for 60 min. The final incubation volume of 100 µL contained a pyrenylbutanamide substrate concentration of 100 µM. The enzyme reaction was terminated by the addition of 200 µL acetonitrile/methanol (1:1, v/v), which contained the internal standard 6-pyren-1-ylhexanoic acid (0.025 µg/200 µL). After cooling in an ice bath for 10 min, the samples were centrifuged at 2000 g at 4 °C for 5 min. Blank incubations in the absence of the enzyme were carried out in parallel.

*HPLC analysis*

The HPLC system consisted of a Bischoff HPLC-compact pump model 2250, a Midas Cool Autosampler, a Bischoff Chromatography column oven and a Waters fluorescence detector model 2475. Data analysis was carried out using a McDacq[32] control chromatography software from Bischoff. Separation was achieved on a Nucleosil 100 C18 analytical column (3 mm inside diameter x 125 mm, particle size 3 µm) (Macherey & Nagel) protected with a Phenomenex C18 guard column (3 mm inside diameter x 4 mm). 50 µL of each sample were injected into the HPLC system. The mobile phase consisted of methanol/water/trifluoroacetic acid (80:20:0.1, v/v/v). Injector temperature was maintained at 10 °C, oven temperature at 20 °C. The flow rate was 0.4 mL/min. The fluorescence detector was set at an excitation wavelength of 340 nm and emission was monitored at 380 nm. To eliminate the large substrate peak in the chromatogram occurring between 3.0 min and 6.3 min and to protect the photomultiplier of the detector from damage by intense light, from 3.0 – 6.3 min excitation wavelength was set to 400 nm and emission wavelength to 600 nm. After 7.0 min an auto zero was carried out.

For calculation of enzyme inhibition the peak ratio of enzyme product and internal standard obtained in presence of a test compound was compared with the mean level of this peak ratio determined in absence of test compounds (= control tests, n = 3). The $IC_{50}$-values were calculated with the aid of Probit transformation.

Under these conditions for the reference inhibitor cyclohexylcarbamic acid 3'-carbamoylbiphenyl-3-ylester (URB 597) (Cayman Chemical) an $IC_{50}$ value of 0.060 ± 0.0067 µM (mean ± standard deviation, n = 4) and for the reference inhibitor 1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenylhexan-1-one (PHOP) (Cayman Chemical) an $IC_{50}$ of 0.0029 ± 0.00049 µM (mean ± standard deviation, n = 4) was measured. These $IC_{50}$ values vary from the data published for these substances recently[1-3] due to the slightly different reaction conditions. While in the first publication the enzyme reaction was performed in Tris buffer for 60 min,[1] in a second publication an incubation time in Tris buffer of only 45 min was used[2] and in a third publication an incubation time in PBS buffer of only 45 min was used.[3] Now a PBS-buffer was used applying an incubation time of 60 min.

## 2.2. MAGL inhibition assay

*Incubation procedure*

An aliquot of a solution of human recombinant MAGL (10 µg/50 µL) (Cayman Chemical) was diluted 1:100 with HEPES buffer (50 mM, pH 7.4 pH at 20 °C) containing 100 mM NaCl, 5 mM $MgCl_2$, 0.1% (m/v) Triton X-100 and 25% (m/v) glycerol. Applying this enzyme solution, in the controls the peak area of the enzyme product 4-pyren-1-ylbutanoic acid amounted to about 70% - 100% of the peak area of the internal standard 6-pyren-1-ylhexanoic acid.

To 2 µL of a DMSO solution of inhibitor or to 2 µL of DMSO in case of the controls was added 91 µL of HEPES buffer (50 mM, pH 7.0 at 20 °C) containing 1 mM EDTA and 0.2% (m/v) Triton X-100. After addition of 2 µL of a solution of the substrate 1,3-dihydroxypropan-1-yl 4-pyren-1-ylbutanoate[4] in DMSO (5 mM), the mixture was pre-incubated at 37 °C for exactly 15 min. Then the enzymatic reaction was started by adding of 5 µL enzyme solution and continued for 45 min at 37 °C. The final incubation volume of 100 µL contained 100 µM of the substrate and 10 ng MAGL. The enzyme reaction was terminated by the addition of 200 µL acetonitrile/methanol (1:1, v/v) spiked with the internal standard 6-pyren-1-ylhexanoic acid (0.10 µg/200 µL). After cooling in an ice bath for 10 min, the samples were centrifuged at 2000 x g and 4 °C for 5 min and stored at -20 °C until HPLC analysis. Blank incubations in the absence of the enzyme were carried out in parallel.

*HPLC analysis*

The HPLC system and the separation conditions were the same as for the measurement of FAAH inhibition, with the exception that only 5 µL of each sample were injected. For calculation of enzyme inhibition the peak ratio of enzyme product 4-pyren-1-ylbutanoic acid and internal standard 6-pyren-1ylhexanoic acid obtained in presence of a test compound was compared with the mean level of this peak ratio determined in absence of test compounds (= control tests, n = 3). Under these conditions for the reference inhibitor [4-(5-methoxy-2-oxo-1,3,4-oxadiazol-3-yl)-2-methylphenyl]carbamic acid benzyl ester (CAY10499) (Cayman Chemical) an $IC_{50}$ values 0.48 ± 0.019 µM (mean ± standard deviation, n = 4) was measured.

### 2.3. Inhibition of cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$)

Inhibition of $cPLA_2\alpha$ was measured according to a recently published procedure.[5] Briefly, $cPLA_2\alpha$ isolated from porcine platelets was incubated with co-vesicles consisting of the substrate 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (200 µM) and 1,2-dioleoyl-*sn*-glycerol (100 µM). Enzyme reactions were terminated after 60 min and $cPLA_2\alpha$ activity was determined by measuring the arachidonic acid released by the enzyme in absence and presence of a test compound with reversed phase HPLC and UV-detection at 200 nm after on-line solid phase extraction.

### 2.4. Metabolic stability in rat liver S9 fractions

The metabolic stability was tested using S9 fractions of rat liver homogenate.[5] Briefly, test compounds were incubated under aerobic conditions in absence and presence of the co-factor NADPH. The metabolic reactions were terminated after 30 min. The extent of metabolism was evaluated with reversed phase HPLC and UV-detection.

The HPLC/UV system consisted of an Ultimate 3000 RS Dionex system. Separation was achieved on a Kromasil C18 analytical column (3 mm inside diameter x 125 mm, particle size 3 µm) protected with a Phenomenex (Aschaffenburg, Germany) C18 guard column (3 mm inside diameter x 4 mm). 30 µL of each sample were injected onto the HPLC system. The temperature of the autosampler was kept at 10 $^{o}$C, the column oven was maintained at 20 $^{o}$C. A gradient method was used starting with 90 % solvent A (acetonitrile/water/concentrated phosphoric acid (85%) 10:90:0.1, v/v/v) and 10 % solvent B (acetonitrile/water/concentrated phosphoric acid (85%) 90:10:0.1, v/v/v) and holding this mixture for 2 min. Then B was linearly increased to 90 % within 9 min and held for 6 min. Finally, the gradient was linearly decreased to 10 % B over 1 min and held for 2 minutes. The flow rate was 0.4 mL/min. The effluents were monitored at 220 nm.

### 2.5. Stability in porcine blood plasma

Porcine plasma was obtained by centrifugation of heparinized porcine blood (20 I.U. heparin/mL) at 1500 x g for 15 min. The plasma was stored at -20 $^{o}$C until used.

*Incubation procedure:* To 125 µL porcine plasma was added 124 µL phosphate buffered saline (prepared from tablets from SigmaAldrich, P4417: one tablet dissolved in 200 mL of deionized water yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 $^{o}$C) followed by 1 µL of a DMSO solution (5 mM) of the test compound. After incubation at 37 $^{o}$C for 30 min, 500 µL acetonitrile was added. The mixture was vortexed and allowed to stand in an ice bath for 15 min. After vigorous vortexing, the mixture was centrifuged at 2000 x g and 4 $^{o}$C for 5 min. The supernatant of the sample was separated and subjected to HPLC, which was performed as described in 2.4.

A test substance blank was prepared by adding 1 µL of a DMSO solution (5 mM) of the test compound to a mixture of 125 mL porcine plasma, 124 µL phosphate buffered saline and 500 µL of acetonitrile. The mixture was vortexed and allowed to stand at room temperature for 30 min and in an ice bath for 15 min. After vigorous vortexing, the mixture was centrifuged at 2000 x g and 4 $^{o}$C for 5 min. The supernatant of the sample was separated and subjected to HPLC, which was performed as described in 2.4.

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