Reversing binding sensitivity to A147T translocator protein

Supplementary Information

1.1 General Experimental

Dichloromethane and triethylamine were distilled from calcium hydride. Anhydrous DMF, methanol, THF, and acetonitrile were obtained from a PureSolv MD 7 solvent purification system (Innovative Technology, Inc.). Potassium carbonate was dried at 150 °C for 16 h prior to use. All other solvents and reagents were used as received from commercial sources. Microwave-assisted reactions were performed in sealed-tube systems using Discover® S-Class (Ai Scientific CEM Corporation).

Melting points were measured in open capillaries using a Stanford Research Systems Optimelt Automated melting point apparatus. IR spectra were acquired neat using a Bruker ALPHA FT-IR spectrometer. Absorption maxima are expressed in wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded at 300 K on Bruker Avance DRX 300, DRX 400, or AVANCE III 500 Ascend spectrometers (^{1}H frequencies 300, 400, 500 MHz; ^{13}C frequencies 75, 100 and 125 MHz respectively). ^{1}H chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 7.26), dimethyl sulfoxide (δ 2.50), methanol (δ 3.31) or acetone (δ 2.05) as reference and are reported as chemical shift (δ_H); relative integral; multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, td = triplet of doublets, q = quartet, m = multiplet, bm = broad multiplet); coupling constants (*J*) reported in Hz; assignment. ^{13}C chemical shifts are expressed as parts per million (ppm) with residual chloroform (δ 77.16), dimethyl sulfoxide (δ 39.52), methanol (δ 49.00) or acetone (δ 29.84) as reference and are reported as chemical shift (δ_C); multiplicity. Assignment of signals was assisted by COSY, edited HSQC, HMBC and DEPT experiments where necessary.

Elemental microanalysis was performed by the Chemical Analysis Facility at the Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia. Low- and high-resolution mass spectra were obtained through either electrospray ionisation (ESI) or atmospheric pressure chemical ionisation (APCI). Low-resolution mass spectra were run on a Finnigan LCQ mass spectrometer. High-resolution mass spectra were run on a Bruker 7T Apex Qe Fourier Transform Ion Cyclotron resonance mass spectrometer equipped with an

Apollo II ESI/APCI/MALDI Dual source. Samples run by ESI were directly infused (150 µL/h) using a Cole Palmer syringe pump. Samples run by APCI were injected (5 µL) into a flow of methanol (0.3 mL/min) by HPLC (Agilent 1100) with no column and coupled to the mass spectrometer.

Analytical HPLC traces were taken on a Waters 2695 Separations module equipped with the Waters Alliance Series Column Heater (set at 30 °C) and Waters 2489 UV/Visible Detector (set at 230 nm and 254 nm). All samples were eluted through a Waters XBridge® C18 5 µm column (2.1×150 mm) using a flow rate of 0.2 mL/min of Solvent A: MilliQ water (+0.1% trifluoroacetic acid) and Solvent B: acetonitrile (+0.1% trifluoroacetic acid). The method consisted of 20% Solvent B for 1 min, then a constant gradient to 100% Solvent B over 25 min. Data acquisition and processing was performed with the Waters Empower 2 software. Reported data for all compounds are based on the 254 nm channel.

1.2 Synthesis

2-(Trichloromethyl)-1*H*-benzo[*d*]imidazole (29)

Methyl 2,2,2-trichloroacetimidate (1.05 equiv.) was added to a suspension of benzene-1,2-diamine (2.009 g, 18.59 mmol, 1.00 equiv.) in acetic acid (3 mL/mmol of diamine) and the reaction mixture was stirred at room temperature for 1 h. Water was added and the resultant precipitate was collected. The crude product was recrystallized from acetic acid to give **29** (3.102 g, 13.2 mmol, 71%) as a colorless solid; mp >300 °C (from dichloromethane); R_f 0.61 (10% methanol in dichloromethane); δ_H (300 MHz; $(CD_3)_2SO$) 7.68–7.65 (2 H, m), 7.37–7.31 (2 H, m); *m*/*z* (+ESI) 239/237/235 ($[M+H]^+$, 32/95/100%), 223 (93), 191 (49).

General Procedure A: Synthesis of Benzimidazole-2-carboxamides From 2-(Trichloromethyl)benzimidazoles

To a suspension of 2-(trichloromethyl)benzimidazole (1.0 equiv.) in acetonitrile (7 mL/mmol of benzimidazole) and water (3.5 mL/mmol of benzimidazole) at 0 °C was added the required secondary amine (1.2–5.0 equiv.) and potassium carbonate solution (4 M, 0.7 mL/mmol of benzimidazole). The reaction mixture was warmed to room temperature and stirred for 16–24 h. The acetonitrile was removed *in vacuo* and the aqueous layer was diluted with an equal

volume of water. The reaction mixture was extracted thrice with ethyl acetate, and the combined organic layers were washed with sodium carbonate, brine and dried (anhydrous $MgSO_4$). The solvent was removed *in vacuo* to give the crude amides that were purified by column chromatography.

_N_,_N_-Diethyl-1_H_-benzo[_d_]imidazole-2-carboxamide (30)

General Procedure A was followed with **29** (2.011 g, 8.539 mmol, 1.00 equiv.) and diethylamine (1.759 mL, 17.08 mmol, 2.000 equiv.). Column chromatography (3% methanol in dichloromethane) gave **30** (1.024 g, 4.713 mmol, 55%) as a colorless solid; mp 124.3–125.7 °C (from dichloromethane); R_f 0.61 (10% methanol in dichloromethane); (Found: C, 66.3; H, 6.9; N, 19.3. $C_{12}H_{15}N_3O$ requires C, 66.3; H, 7.0; N, 19.3%); ν_{max} (neat)/cm^{-1} 3216 (b, NH), 2933, 2871, 1611, 1584, 1519, 1403, 1318, 1278, 1087, 789, 739; δ_H (300 MHz; $(CD_3)_2SO$) 13.04 (1 H, bs), 7.62 (2 H, bs), 7.28–7.25 (2 H, m), 4.09 (2 H, q, *J* 7.0 Hz), 3.51 (2 H, q, *J* 7.1 Hz), 1.24 (3 H, t, *J* 7.0 Hz), 1.18 (3 H, t, *J* 7.1 Hz); δ_C (100 MHz; $(CD_3)_2SO$) 159.1 (C), 145.9 (C), 142.7 (b, C), 133.4 (b, C), 124.0 (b, CH), 122.3 (b, CH), 120.2 (b, CH), 112.2 (b, CH), 42.5 (CH_2), 40.8 (CH_2), 14.6 (CH_3), 12.7 (CH_3); *m*/*z* (−ESI) 455 (17%), 284 (19), 216 ($[M-H]^-$, 100).

_N_-Methyl-_N_-(prop-2-yn-1-yl)-1_H_-benzo[_d_]imidazole-2-carboxamide (31)

General Procedure A was followed with **29** (1.021 g, 4.335 mmol, 1.000 equiv.) and *N*-methylpropargylamine (430 μL, 5.10 mmol, 1.18 equiv.). Column chromatography (15% ethyl acetate in hexane) gave **31** (488 mg, 2.29 mmol, 53%) as a colorless solid; mp 147.0–148.5 °C (from dichloromethane); R_f 0.36 (30% ethyl acetate in hexane); (Found: C, 67.1; H, 5.3; N, 19.5. $C_{12}H_{11}N_3O$ requires C, 67.6; H, 5.2; N, 19.7%); (Found (+ESI): $[M+Na]^+$, 236.0794. $C_{12}H_{11}N_3O$+Na requires 236.0794); ν_{max} (neat)/cm^{-1} 3244 (b, NH/propargyl CH), 2923, 2851, 1621 (CO), 1526, 1399, 1247, 996, 747; δ_H (400 MHz; $(CD_3)_2CO$) 12.20 (1 H, bs), 7.79–7.78 (1 H, m), 7.66–7.64 (1 H, m), 7.38–7.31 (2 H, m), 5.53 (1.1 H, d, *J* 2.4 Hz), 4.46 (0.9 H, d, *J* 2.5 Hz), 3.97 (1.3 H, s), 3.20 (1.7 H, s), 2.84–2.82 (1 H, m); δ_C (100 MHz; $(CD_3)_2CO$) 160.1 (C), 159.6 (C), 146.4 (C), 146.3 (C), 144.2 (C), 144.1 (C), 134.3 (C), 134.2 (C), 125.6 (CH), 125.5 (CH), 123.6 (CH), 123.5 (CH), 121.6 (CH), 121.5 (CH), 113.1 (CH), 113.0 (CH), 80.2 (C), 79.5 (C), 74.0 (CH),

73.6 (CH), 40.4 (CH_2), 38.0 (CH_2), 36.4 (CH_3), 34.2 (CH_3); *m*/*z* (−ESI) 357 (35%), 213 (43), 212 ($[M-H]^-$, 100), 127 (36).

_N_-Benzyl-_N_-methyl-1_H_-benzo[_d_]imidazole-2-carboxamide (32)

General Procedure A was followed with **29** (1.007 g, 4.276 mmol, 1.000 equiv.) and *N*-benzylmethylamine (1.10 mL, 8.52 mmol, 1.99 equiv.). Column chromatography (30% ethyl acetate in hexane) gave **32** (1.086 g, 4.093 mmol, 96%) as a colorless solid; mp 143.1–146.6 °C (from dichloromethane); R_f 0.48 (50% ethyl acetate in hexane); (Found: C, 72.0; H, 5.8; N, 15.5. $C_{16}H_{15}N_3O$ requires C, 72.4 H, 5.7; N, 15.8%); (Found (+ESI): $[M+Na]^+$, 288.1108. $C_{16}H_{15}N_3O$+Na requires 288.1107); ν_{max} (neat)/cm^{-1} 3288 (b, NH), 2924, 1615 (CO), 1523, 1495, 1439, 1399, 1314, 1243, 745; δ_H (300 MHz; $(CD_3)_2SO$) 13.21 (1 H, bs), 7.65–7.62 (2 H, m), 7.43–7.27 (7 H, m), 5.57 (1.0 H, s), 4.77 (1.1 H, s), 3.56 (1.4 H, s), 2.96 (1.5 H, s); δ_C (75 MHz; $(CD_3)_2SO$) 159.9 (C), 159.7 (C), 145.69 (C), 145.66 (C), 137.5 (C), 137.0 (C), 128.6 (CH), 127.7 (CH), 127.4 (CH), 127.33 (CH), 127.31 (CH), 123.33 (CH), 53.0 (CH_2), 51.2 (CH_2), 36.5 (CH_3), 33.9 (CH_3); *m*/*z* (−ESI) 264 ($[M-H]^-$, 100).

General procedure B: _N_-Benzylation/Alkylation of Indoles and Benzimidazoles

Sodium hydride (60% w/w dispersion in mineral oil, 1.20 equiv. unless otherwise stated) was added in parts to a solution of indole or benzimidazole (1.00 equiv.) in DMF (2 mL/mmol of indole/benzimidazole) at 0 °C and the reaction mixture was stirred for 30 min at room temperature. The required benzylating or alkylating agent (0.94–1.50 equiv.) was added at 0 °C and the mixture was warmed to room temperature and stirred for 2–24 h until complete by TLC. The reaction mixture was poured into ice water, neutralised with aqueous hydrochloric acid (1 M) and extracted thrice with ethyl acetate. The organic layers were combined, washed thrice with water and once with brine, dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo*. Column chromatography gave the desired *N*-benzylated or *N*-alkylated products.

1-Benzyl-_N_,_N_-diethyl-1_H_-benzo[_d_]imidazole-2-carboxamide (4)

General Procedure B was followed with **30** (92 mg, 0.34 mmol, 1.00 equiv.) and benzyl bromide (60 μL, 0.51 mmol, 1.5 equiv.). Column chromatography (30% ethyl acetate in hexane) gave **4** (102 mg, 0.332 mmol, 98%) as a colorless solid; mp 108.7–109.6 °C (from dichloromethane); R_f 0.31 (30% ethyl acetate in hexane); (Found: C, 74.0; H, 6.9; N, 19.3. $C_{19}H_{21}N_3O$ requires C, 74.2; H, 6.9; N, 19.3%); (Found (+ESI): $[M+Na]^+$, 330.1576. $C_{19}H_{21}N_3O$+Na requires 330.1577); ν_{max} (neat)/cm^{-1} 2965, 2929, 1630 (CO), 1514, 1453, 1426, 1274, 1215, 1121, 880, 743; δ_H (300 MHz; $CDCl_3$) 7.83–7.80 (1 H, m), 7.42–7.39 (1 H, m), 7.33–7.19 (7 H, m), 5.56 (2 H, s), 3.52 (2 H, q, *J* 7.1 Hz), 3.47 (2 H, q, *J* 7.3 Hz), 1.19 (3 H, t, *J* 7.1 Hz), 1.00 (3 H, t, *J* 7.2 Hz); δ_C (75 MHz; $CDCl_3$) 161.4 (C), 146.1 (C), 141.8 (C), 136.7 (C), 135.0 (C), 128.9 (CH), 128.1 (CH), 127.5 (CH), 124.2 (CH), 123.0 (CH), 120.9 (CH), 110.5 (CH), 48.1 (CH_2), 43.4 (CH_2), 40.2 (CH_2), 14.3 (CH_3), 12.7 (CH_3); *m*/*z* (+ESI) 638 (51%), 637 ($[2M+Na]^+$, 100), 481 (34), 370 ($[M+Na]^+$, 32), 308 ($[M+H]^+$, 16).

_N_,_N_-Diethyl-1-(4-methoxybenzyl)-1_H_-benzo[_d_]imidazole-2-carboxamide (5)

General Procedure B was followed with **30** (71 mg, 0.33 mmol, 1.00 equiv.) and 4-methoxybenzyl chloride (50 μL, 0.37 mmol, 1.12 equiv.). Column chromatography (25% ethyl acetate in hexane) gave **5** (102 mg, 0.302 mmol, 92%) as a colorless gummy mass; R_f 0.37 (50% ethyl acetate in hexane; (Found (+ESI): $[M+Na]^+$, 360.1684. $C_{20}H_{23}N_3O_2$+Na requires 360.1683); ν_{max} (neat)/cm^{-1} 2973, 2936, 2836, 1632 (CO), 1513, 1455, 1276, 1249, 1177, 1033, 742; δ_H (300 MHz; $CDCl_3$) 7.82–7.80 (1 H, m), 7.44–7.38 (1 H, m), 7.35–7.28 (2 H, m), 7.16 (2 H, d, *J* 8.7 Hz), 6.79 (2 H, d, *J* 8.7 Hz), 5.47 (2 H, s), 3.75 (3 H, s), 3.54 (2 H, q, *J* 7.1 Hz), 3.46 (2 H, q, *J* 7.1 Hz), 1.22 (3 H, t, *J* 7.1 Hz), 1.01 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 161.4 (C), 159.5 (C), 146.0 (C), 141.6 (C), 134.9 (C), 129.0 (CH), 128.8 (C), 124.2 (CH), 123.0 (CH), 120.8 (CH), 114.2 (CH), 110.6 (CH), 55.4 (CH_3), 47.6 (CH_2), 43.4 (CH_2), 40.2 (CH_2), 14.3 (CH_3), 12.8 (CH_3); *m*/*z* (+ESI) 697 ($[2M+Na]^+$, 67%), 675 ($[2M+H]^+$, 91), 654 (19), 338 ($[M+H]^+$, 100).

1-(4-Chlorobenzyl)-_N_,_N_-diethyl-1_H_-benzo[_d_]imidazole-2-carboxamide (6)

General Procedure B was followed with **30** (120 mg, 0.553 mmol, 1.00 equiv.) and 4-chlorobenzyl chloride (116 mg, 0.720 mmol, 1.30 equiv.). Column chromatography (30% ethyl

acetate in hexane) gave **6** (183 mg, 0.535 mmol, 97%) as a colorless solid; mp 70.8–72.2 °C (from dichloromethane); R_f 0.35 (50% ethyl acetate in hexane); (Found: C, 66.7; H, 5.8; N, 12.3. $C_{19}H_{20}N_3OCl$ requires C, 66.8; H, 5.9; N, 12.2%); (Found (+ESI): $[M+Na]^+$, 364.1187. $C_{19}H_{20}N_3O^{35}Cl$+Na requires 364.1187); ν_{max} (neat)/cm^{-1} 2927, 2871, 1630 (CO), 1492, 1454, 1381, 1272, 1157, 1095, 1016, 880, 741; δ_H (300 MHz; $CDCl_3$) 7.83–7.79 (1 H, m), 7.37–7.30 (3 H, m), 7.25 (2 H, d, *J* 8.4 Hz), 7.15 (2 H, d, *J* 8.4 Hz), 5.52 (2 H, s), 3.55 (2 H, q, *J* 7.0 Hz), 3.53 (2 H, t, *J* 7.1 Hz), 1.21 (3 H, t, *J* 7.1 Hz), 1.07 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 161.2 (C), 145.9 (C), 141.8 (C), 135.2 (C), 134.8 (C), 133.9 (C), 129.0 (CH), 128.9 (CH), 124.4 (CH), 123.1 (CH), 121.0 (CH), 110.4 (CH), 47.6 (CH_2), 43.5 (CH_2), 40.3 (CH_2), 14.4 (CH_3), 12.8 (CH_3); *m*/*z* (+ESI) 709/707/705 ($[2M+Na]^+$, 29/66/100%), 682 ($[2M]^+$, 26), 366/364 ($[M+Na]^+$, 7/13), 344/342 ($[M+H]^+$, 17/40).

_N_,_N_-Diethyl-1-((6-methoxypyridin-3-yl)methyl)-1_H_-benzo[_d_]imidazole-2-carboxamide (7)

General Procedure B was followed with **30** (101 mg, 0.465 mmol, 1.00 equiv.) and 5-(chloromethyl)-2-methoxypyridine (92 mg, 0.584 mmol, 1.26 equiv.). Column chromatography (30–40% ethyl acetate in hexane) gave **7** (149 mg, 0.440 mmol, 95%) as a colorless oil; R_f 0.17 (50% ethyl acetate in hexane); (Found (+ESI): $[M+Na]^+$, 361.1635. $C_{19}H_{22}N_4O_2$+Na requires 361.1635); ν_{max} (neat)/cm^{-1} 2973, 2944, 1634 (CO), 1612, 1495, 1455, 1310, 1279, 1025, 744; δ_H (300 MHz; $CDCl_3$) 8.13 (1 H, d, *J* 1.8 Hz), 7.81–7.78 (1 H, m), 7.52 (1 H, dd, *J* 8.6, 2.3 Hz), 7.41–7.38 (1 H, m), 7.34–7.27 (2 H, m), 6.64 (1 H, d, *J* 8.4 Hz), 5.47 (2 H, s), 3.88 (3 H, s), 3.60–3.52 (4 H, m), 1.24 (3 H, t, *J* 7.1 Hz), 1.06 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 164.1 (C), 161.2 (C), 146.1 (CH), 145.8 (C), 141.8 (C), 138.5 (CH), 134.7 (C), 125.1 (C), 124.4 (CH), 123.1 (CH), 121.0 (CH), 111.4 (CH), 110.4 (CH), 53.6 (CH_3), 45.4 (CH_2), 43.6 (CH_2), 40.4 (CH_2), 14.5 (CH_3), 12.8 (CH_3); *m*/*z* (+ESI) 700 (34%), 699 ($[2M+Na]^+$, 100), 677 ($[2M+H]^+$, 36), 401 (16), 339 ($[M+H]^+$, 9).

_N_-Benzyl-1-(4-chlorobenzyl)-_N_-methyl-1_H_-benzo[_d_]imidazole-2-carboxamide (8)

General Procedure B was followed with **32** (150 mg, 0.565 mmol, 1.00 equiv.) and 4-chlorobenzyl chloride (113 mg, 0.702 mmol, 1.24 equiv.). Column chromatography (30% ethyl

acetate in hexane) gave **8** (200 mg, 0.513 mmol, 91%) as a colorless gummy mass; R_f 0.18 (30% ethyl acetate in hexane); (Found (+ESI): $[M+Na]^+$, 412.1187. $C_{23}H_{20}N_3O^{35}Cl$+Na requires 412.1187); ν_{max} (neat)/cm^{-1} 3030, 2927, 1633 (CO), 1492, 1453, 1383, 1247, 1086, 1015, 820, 736; δ_H (400 MHz; $CDCl_3$) 7.89–7.83 (1 H, m), 7.41–7.22 (9 H, m), 7.16 (1.0 H, d, *J* 8.5 Hz), 7.13 (1.0 H, d, *J* 8.5 Hz), 7.00–6.98 (1 H, m), 5.64–5.63 (2 H, 2×s), 5.02 (1.0 H, s), 4.76 (1.1 H, s), 3.13 (1.6 H, s), 3.03 (1.4 H, s); δ_C (100 MHz; $CDCl_3$) 161.8 (C), 161.6 (C), 145.1 (C), 144.9 (C), 141.62 (C), 141.57 (C), 136.4 (C), 136.1 (C), 135.2 (C), 135.1 (C), 135.0 (C), 134.9 (C), 133.90 (C), 133.88 (C), 129.13 (CH), 129.09 (CH), 128.9 (CH), 128.8 (2×CH), 128.5 (CH), 128.2 (CH), 127.80 (CH), 127.79 (CH), 127.4 (CH), 124.71 (CH), 124.68 (CH), 123.37 (CH), 123.36 (CH), 121.1 (CH), 121.0 (CH), 110.5 (CH), 110.4 (CH), 54.9 (CH_2), 51.1 (CH_2), 47.63 (CH_2), 47.59 (CH_2), 36.6 (CH_3), 33.5 (CH_3); *m*/*z* (+ESI) 803/801 ($[2M+Na]^+$, 47/89%), 802 (100), 781/779 ($[2M+H]^+$, 18/29), 693, 390/392 (9/35).

General Procedure C: Alternate Procedure for *N*-Benzylation of Benzimidazoles

To a solution of benzimidazole (1.00 equiv.) in acetone (2 mL/mmol of benzimidazole) was added potassium carbonate (5.00 equiv.) and benzylating agent (1.23–1.46 equiv.). The reaction mixture was heated under reflux for 24 h, filtered, and the solvent was removed *in vacuo*. Column chromatography gave the desired *N*-benzylbenzimidazoles.

1-(4-Methoxybenzyl)-*N*-methyl-*N*-(prop-2-yn-1-yl)-1*H*-benzo[*d*]imidazole-2-carboxamide (9)

General Procedure C was followed with **31** (82 mg, 0.385 mmol, 1.00 equiv.) and 4-methoxybenzyl chloride (76 μL, 0.561 mmol, 1.46 equiv.). Column chromatography (30–35% ethyl acetate in hexane) gave **9** (75 mg, 0.22 mmol, 57%) as a colorless solid and mix of rotamers in NMR; mp 110.6–113.5 °C (from dichloromethane); R_f 0.23 (30% ethyl acetate in hexane); (Found: C, 72.4; H, 6.0; N, 12.5. $C_{20}H_{19}N_3O_2$ requires C, 72.1; H, 5.7; N, 12.6%); (Found (+APCI): $[M+H]^+$, 334.1550. $C_{20}H_{19}N_3O_2$+H requires 334.1550); ν_{max} (neat)/cm^{-1} 3290 (propargyl CH), 2931, 2837, 1639 (CO), 1455, 1422, 1246, 1176, 1086, 1031, 738; δ_H (400 MHz; $CDCl_3$) 7.83–7.80 (1 H, m), 7.44–7.42 (0.5 H, m), 7.39–7.37 (0.5 H, m), 7.35–7.29 (2 H, m), 7.17

(2 H, d, *J* 8.7 Hz), 6.80 (2 H, d, *J* 8.7 Hz), 5.54 (1.0 H, s), 5.50 (1.1 H, s), 4.62 (0.9 H, d, *J* 2.5 Hz), 4.37 (1.1 H, d, *J* 2.5 Hz), 3.75 (3 H, s), 3.20–3.18 (3 H, 2×s), 2.27 (0.5 H, t, *J* 2.5), 2.21 (0.4 H, t, *J* 2.4,); δ_C (100 MHz; $CDCl_3$) 161.6 (C), 161.4 (C), 159.4 (C), 159.3 (C), 144.8 (C), 144.5 (C), 141.6 (C), 135.0 (C), 134.8 (C), 128.9 (CH), 128.8 (CH), 128.6 (C), 128.5 (C), 124.6 (CH), 123.31 (CH), 123.28 (CH), 121.0 (CH), 120.9 (CH), 114.29 (CH), 114.25 (CH), 111.0 (CH), 110.8 (CH), 78.2 (C), 77.8 (C), 73.1 (CH), 72.6 (CH), 55.38 (CH_3), 55.37 (CH_3), 48.1 (CH_2), 47.8 (CH_2), 40.9 (CH_2), 36.7 (CH_2), 36.3 (CH_3), 33.2 (CH_3); *m/z* (+ESI) 704 (21%), 690 (46), 689 ($[2M+Na]^+$, 100), 356 ($[M+Na]^+$, 11), 334 ($[M+H]^+$, 5).

1-(4-Chlorobenzyl)-*N*-methyl-*N*-(prop-2-yn-1-yl)-1*H*-benzo[*d*]imidazole-2-carboxamide (10)

General Procedure C was followed with **31** (121 mg, 0.567 mmol, 1.00 equiv.) and 4-chlorobenzyl chloride (112 mg, 0.696 mmol, 1.23 equiv.) Column chromatography (10–15% ethyl acetate in hexane) gave **10** (151 mg, 0.447 mmol, 79%) as a colorless solid; mp 105.3–107.9 °C (from dichloromethane); R_f 0.28 (30% ethyl acetate in hexane); (Found: C, 67.3; H, 4.9; N, 12.4. $C_{19}H_{16}N_3OCl$ requires C, 67.6; H, 4.8; N, 12.4%); (Found (+APCI): $[M+H]^+$, 338.1056. $C_{19}H_{16}N_3O^{35}Cl$+H requires 338.1055); ν_{max} (neat)/cm^{-1} 3294 (propargyl CH), 2926, 1638 (CO), 1492, 1455, 1250, 1087, 1015, 743; δ_H (400 MHz; $CDCl_3$) 7.85–7.81 (1 H, m), 7.37–7.33 (3 H, m), 7.26 (1.0 H, d, *J* 8.5 Hz), 7.25 (1.0 H, d, *J* 8.6 Hz), 7.16 (1.0 H, d, *J* 8.6 Hz), 7.15 (1.0 H, d, *J* 8.5 Hz), 5.59 (1.0 H, s), 5.56 (1.0 H, s), 4.70 (1.0 H, d, *J* 2.4 Hz), 4.36 (1.1 H, d, *J* 2.4 Hz), 3.27 (1.5 H, s), 3.19 (1.4 H, s), 2.27 (0.5 H, t, J 2.5 Hz), 2.21 (0.4 H, t, *J* 2.4 Hz); δ_C (100 MHz; $CDCl_3$) 161.3 (C), 161.2 (C), 144.5 (C), 144.3 (C), 141.5 (C), 135.01 (C), 134.95 (C), 134.9 (C), 134.8 (C), 134.0 (C), 133.9 (C), 129.2 (CH), 129.1 (CH), 128.8 (CH), 128.7 (CH), 124.92 (CH), 124.88 (CH), 123.6 (CH), 123.5 (CH), 121.2 (CH), 121.1 (CH), 110.7 (CH), 110.6 (CH), 78.2 (C), 77.7 (C), 73.1 (CH), 72.6 (CH), 47.9 (CH_2), 47.8 (CH_2), 40.9 (CH_2), 36.8 (CH_2), 36.4 (CH_3), 33.3 (CH_3); *m/z* (+ESI) 701/699/697 ($[2M+Na]^+$, 18/53/100%), 643/641 (25/63), 516 (25), 340/338 ($[M+H]^+$, 5/27).

Ethyl 1-(4-(Benzyloxy)benzyl)-5-methyl-1*H*-indole-2-carboxylate (34)

General Procedure B was followed with ethyl 5-methoxy-1*H*-indole-2-carboxylate (1.001 g, 4.92 mmol, 1.00 equiv.) and 4-(benzyloxy)benzyl chloride (1.718 g, 7.383 mmol, 1.50 equiv.). Column chromatography (3–4% ethyl acetate in hexane) gave **34** (982 mg, 2.46 mmol, 50%) as a colorless solid; mp 87.0–88.1°C (from dichloromethane); R_f 0.63 (20% ethyl acetate in hexane); (Found: C, 78.3; H, 6.2; N, 3.4. $C_{26}H_{25}NO_3$ requires C, 78.2; H, 6.3; N, 3.5%); (Found (+ESI): $[M+Na]^+$, 422.1724. $C_{26}H_{25}NO_3$+Na requires 422.1727); ν_{max} (neat)/cm^{-1} 3032, 2980, 2865, 1701 (CO), 1510, 1455, 1242, 1195, 1174, 1148, 1092, 1023, 739; δ_H (500 MHz; $CDCl_3$) 7.49 (1 H, t, *J* 0.7), 7.41–7.30 (6 H, m), 7.29 (1 H, d, *J* 8.5 Hz), 7.15 (1 H, dd, *J* 8.5, 1.5 Hz), 7.03 (2 H, d, *J* 9.0 Hz), 6.86 (2 H, d, *J* 9.0 Hz), 5.77 (2 H, s), 5.00 (2 H, s), 4.35 (2 H, q, *J* 7.2 Hz), 2.46 (3 H, s), 1.38 (3 H, t, *J* 7.3); δ_C (125 MHz; $CDCl_3$) 162.2 (C), 158.0 (C), 138.1 (C), 137.2 (C), 131.0 (C), 130.2 (C), 128.7 (CH), 128.0 (CH), 127.8 (CH), 127.7 (C), 127.6 (CH), 127.3 (CH), 126.5 (C), 122.0 (CH), 115.0 (CH), 110.7 (CH), 110.5 (CH), 70.1 (CH_2), 60.6 (CH_2), 47.4 (CH_2), 21.5 (CH_3), 14.5 (CH_3); *m/z* (+ESI) 821 ($[2M+Na]^+$, 16%), 815 (54), 417 ($[M+NH_4]^+$, 100), 400 ($[M+H]^+$, 51), 318 (18).

General procedure D: Hydrolysis of Ethyl Esters

Potassium hydroxide pellets (3.0 equiv.) were added to a suspension of carboxylic acid (1.0 equiv.) in ethanol (2.5 mL/mmol of ester) and water (1.25 mL/mmol of ester). The reaction mixture was heated under reflux for 2–4 h until complete by TLC. Solvent was removed *in vacuo* and the resulting potassium salt was dissolved in a minimum volume of water and acidified to pH 3. Filtration gave the desired acids which were dried under high vacuum at 60 °C.

1-(4-(Benzyloxy)benzyl)-5-methyl-1*H*-indole-2-carboxylic Acid (35)

General Procedure D was followed with **34** (584 mg, 1.46 mmol) to give **35** (414 mg, 1.11 mmol, 76%) as a colorless solid; mp 219.3–223.1 °C (from water); R_f 0.82 (10% methanol in dichloromethane); (Found: C, 77.2; H, 5.6; N, 3.5. $C_{24}H_{21}NO_3$ requires C, 77.6; H, 5.7; N, 3.8%); ν_{max} (neat)/cm^{-1} 3500–2200 (COOH), 2875, 1670 (CO), 1510, 1265, 1240, 1211, 1173, 1011, 798, 745; δ_H (300 MHz; $(CD_3)_2SO$) 12.91 (1 H, bs), 7.45–7.30 (7 H, m), 7.20 (1 H, s, H2), 6.99 (2

H, d, *J* 8.4 Hz), 6.89 (2 H, d, *J* 8.1 Hz), 5.76 (2 H, s), 5.01 (2 H, s), 2.36 (3 H, s); δ_C (75 MHz; $(CD_3)_2SO$) 163.0 (C), 157.4 (C), 137.4 (C), 137.0 (C), 130.9 (C), 129.3 (C), 128.4 (CH), 128.0 (C), 127.8 (CH), 127.7 (CH), 127.6 (CH), 126.7 (CH), 125.8 (C), 121.5 (CH), 114.7 (CH), 111.1 (CH), 109.8 (CH), 69.2 (CH_2), 46.3 (CH_2), 29.9 (CH_3); *m*/*z* (−ESI) 741 ([2M−H]$^-$, 31%), 371 (26), 370 ([M−H]$^-$, 100).

General Procedure E: Amide Coupling *via* Acid Chloride Intermediates

Oxalyl chloride (4.0 equiv.) was added dropwise to a solution of carboxylic acid (1.0 equiv.) in dichloromethane (2.5 mL/mmol of acid) at 0 °C. Several drops of DMF were added and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed (N_2 stream, high vacuum) and the acid chloride was redissolved in dichloromethane (2.5 mL/mmol of acid chloride). *N*-methylpropargylamine (1.2–1.5 equiv.) or diethylamine (3.0 equiv.) was added at 0 °C, followed by triethylamine (3.0 equiv.), and the reaction mixture was warmed to room temperature and stirred for 3–16 h until complete by TLC. The reaction mixture was diluted with dichloromethane and washed with water. The aqueous layer was re-extracted twice with dichloromethane and the combined organic layers were washed with saturated sodium bicarbonate solution, aqueous hydrochloric acid (1 M) and brine and dried over anhydrous $MgSO_4$. Solvents were removed *in vacuo* and the crude product was purified by column chromatography to give the desired amides.

1-(4-(Benzyloxy)benzyl)-*N*,*N*-diethyl-5-methyl-1*H*-indole-2-carboxamide (11)

General Procedure E was followed with **35** (700 mg, 1.89 mmol, 1.00 equiv.) and diethylamine (582 µL, 5.65 mmol, 2.99 equiv.). Column chromatography (15–20% ethyl acetate in hexane) gave **11** (758 mg, 1.78 mmol, 94%) as a colorless gummy mass; R_f 0.21 (20% ethyl acetate in hexane); (Found (+ESI): [M+Na]$^+$, 449.2201. $C_{28}H_{30}N_2O_2$+Na requires 449.2200); ν_{max} (neat)/cm^{-1} 2973, 2933, 2872, 1623 (CO), 1510, 1454, 1270, 1240, 1218, 1174, 1016, 792, 740; δ_H (300 MHz; $CDCl_3$) 7.42–7.29 (7 H, m, H3/H6/H16–H18), 7.09 (1 H, dd, *J* 8.5, 1.2 Hz), 7.04 (2 H, d, *J* 8.7 Hz), 6.83 (2 H, d, *J* 8.7 Hz), 6.54 (1 H, s), 5.40 (2 H, s), 5.01 (2 H, s), 3.32 (4 H, bs), 2.46 (3 H, s), 1.07 (6 H, bs); δ_C (75 MHz; $CDCl_3$) 164.4 (C), 158.1 (C), 137.1 (C), 136.0 (C), 132.8

(C), 130.9 (C), 129.6 (C), 128.7 (CH), 128.5 (CH), 128.0 (CH), 127.5 (CH), 127.0 (C), 125.0 (CH), 121.1 (CH), 115.0 (CH), 109.9 (CH), 102.2 (CH), 70.1 (CH_2), 47.1 (CH_2), 43.0 (b, CH_2), 39.5 (b, CH_2), 21.5 (CH_3), 13.4 (b, CH_3); *m/z* (+ESI) 875 ($[2M+Na]^+$, 25%), 870 ($[2M+NH_4]^+$, 58), 853 ($[2M+H]^+$, 19), 528 (17), 427 ($[M+H]^+$, 100).

_N_,_N_-Diethyl-1-(4-hydroxybenzyl)-5-methyl-1_H_-indole-2-carboxamide (12)

Palladium on carbon (177 mg, 51% w/w) was added to a suspension of **11** (347 mg, 0.814 mmol, 1.00 equiv.) in methanol (30 mL). The reaction mixture was stirred under a balloon of hydrogen for 2 h, filtered through a pad of Celite® and the solvent was removed *in vacuo*. Column chromatography (2% methanol in dichloromethane) followed by recrystallisation (ethyl acetate) gave **12** (233 mg, 0.693 mmol, 85%) as a colorless solid; mp 193.0–193.6 °C (from ethyl acetate); R_f 0.44 (50% ethyl acetate in hexane); (Found: C, 74.9; H, 7.2; N, 8.3. $C_{21}H_{24}N_2O_2$ requires C, 75.0; H, 7.2; N, 8.3%); (Found (+ESI): $[M+Na]^+$, 359.1728. $C_{21}H_{24}N_2O_2$+Na requires 359.1730); ν_{max} (neat)/cm^{-1} 3600–3100 (OH), 2972, 2916, 2851, 1606 (CO), 1592, 1537, 1516, 1456, 1341, 1272, 1217, 1172, 795; δ_H (500 MHz; $CDCl_3$:CD_3OD (9:1)): 7.38 (1 H, s), 7.29 (1 H, d, *J* 8.5 Hz), 7.06 (1 H, d, *J* 8.0 Hz), 6.86 (2 H, d, *J* 8.5 Hz), 6.61 (2 H, d, *J* 8.5 Hz), 6.50 (1 H, s), 5.27 (2 H, s), 3.37 (2 H, q, *J* 6.5 Hz), 3.07 (2 H, q, *J* 6.5 Hz), 2.41 (3 H, s), 1.05 (3 H, t, *J* 7.0 Hz), 0.97 (3 H, t, *J* 7.0 Hz); δ_C (75 MHz; $CDCl_3$:CD_3OD (9:1)) 164.9 (C), 156.2 (C), 135.9 (C), 132.4 (C), 129.6 (C), 129.2 (C), 128.4 (CH), 126.8 (C), 125.0 (CH), 120.9 (CH), 115.3 (CH), 109.9 (CH), 102.2 (CH), 47.0 (CH_2), 43.3 (CH_2), 39.4 (CH_2), 21.3 (CH_3), 14.0 (CH_3), 12.4 (CH_3); *m/z* (+ESI) 695 ($[2M+Na]^+$, 33%), 690 ($[2M+NH_4]^+$, 100), 673 ($[M+Na]^+$, 62), 627 (14), 337 ($[M+H]^+$, 76).

Ethyl 5-Methyl-1-(prop-2-yn-1-yl)-1_H_-indole-2-carboxylate (36)

General Procedure B was followed with ethyl 5-methyl-1*H*-indole-2-carboxylate (747 mg, 3.68 mmol, 1.00 equiv.), sodium hydride (60% w/w, 138 mg, 3.45 mmol, 0.938 equiv.) and propargyl bromide (80% w/w, 477 μL, 4.43 mmol, 1.20 equiv.). Column chromatography (4% ethyl acetate in hexane) gave **36** (752 mg, 3.12 mmol, 85%) as a colorless solid; mp 91.0–91.7 °C (from dichloromethane); R_f 0.41 (10% ethyl acetate in hexane); (Found: C, 74.4; H, 6.3; N,

5.8. $C_{15}H_{15}NO_2$ requires C, 74.7; H, 6.3; N, 5.8%); ν_{max} (neat)/cm^{-1} 3242 (propargyl CH), 2996, 2915, 1702 (CO), 1469, 1262, 1204, 1169, 1153, 1097, 763, 738; δ_H (300 MHz; $CDCl_3$) 7.46 (1 H, s), 7.40 (1 H, d, *J* 8.7 Hz), 7.26 (1 H, s), 7.23 (1 H, dd, *J* 8.6, 1.4 Hz), 5.42 (2 H, d, *J* 2.4 Hz), 4.40 (2 H, q, *J* 7.1 Hz), 2.46 (3 H, s), 2.24 (1 H, t, *J* 2.6 Hz), 1.42 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 162.2 (C), 137.6 (C), 130.6 (C), 127.5 (CH), 127.0 (C), 126.6 (C), 122.2 (CH), 111.1 (CH), 110.4 (CH), 79.0 (C), 72.0 (CH), 60.8 (CH_2), 34.0 (CH_2), 21.5 (CH_3), 14.5 (CH_3); *m*/*z* (+ESI) 432 (71%), 381 (59), 318 (49), 267 (48), 242 ($[M+H]^+$, 100), 204 (47).

5-Methyl-1-(prop-2-yn-1-yl)-1*H*-indole-2-carboxylic Acid (37)

General Procedure D was followed with **36** (683 mg, 2.83 mmol) to give **37** (597 mg, 2.80 mmol, 99%) as a colorless solid; mp 194.1–199.2 °C (from water); R_f 0.14 (5% methanol in dichloromethane); (Found: C, 72.1; H, 5.3; N, 6.5. $C_{13}H_{11}NO_2 \cdot 0.2H_2O$ requires C, 72.0; H, 5.3; N, 6.5%); ν_{max} (neat)/cm^{-1} 3290 (propargyl CH), 3200–2300 (COOH), 1666 (CO), 1534, 1459, 1347, 1277, 1216, 915, 793; δ_H (300 MHz; $(CD_3)_2SO$) 13.03 (1 H, bs), 7.51 (1 H, d, *J* 8.4 Hz), 7.46 (1 H, s), 7.21–7.18 (2 H, m), 5.44 (2 H, d, *J* 2.4 Hz), 3.20 (1 H, t, *J* 2.3 Hz), 2.34 (3 H, s); δ_C (75 MHz; $(CD_3)_2SO$) 162.7 (C), 137.1 (C), 129.7 (C), 127.5 (C), 126.9 (CH), 125.9 (C), 121.6 (CH), 110.8 (CH), 110.3 (CH), 79.8 (C), 74.3 (CH), 33.4 (CH_2), 21.0 (CH_3); *m*/*z* (−ESI) 213 (16%), 212 ($[M-H]^-$, 100), 168 (7).

***N*,*N*-Diethyl-5-methyl-1-(prop-2-yn-1-yl)-1*H*-indole-2-carboxamide (38)**

General Procedure E was followed with **37** (504 mg, 2.36 mmol, 1.00 equiv.) and diethylamine (725 μL, 7.04 mmol, 2.98 equiv.). Column chromatography (10–15% ethyl acetate in hexane) gave **38** (552 mg, 2.06 mmol, 87%) as a colorless solid; mp 103.9–104.7 °C (from dichloromethane); R_f 0.37 (30% ethyl acetate in hexane); (Found: C, 76.0; H, 7.4; N, 10.4. $C_{17}H_{20}N_2O$ requires C, 76.1; H, 7.5; N, 10.4%); ν_{max} (neat)/cm^{-1} 3286 (propargyl CH), 3233 (propargyl CH), 2973, 2934, 1618 (CO), 1534, 1455, 1424, 1341, 1272, 1218, 1166, 1137, 1089, 792; δ_H (300 MHz; $CDCl_3$) 7.41 (1 H, s), 7.35 (1 H, d, *J* 8.4 Hz), 7.14 (1 H, dd, *J* 8.4, 1.5 Hz), 6.57 (1 H, s), 5.06 (2 H, d, *J* 2.7 Hz), 3.58 (4 H, q, *J* 7.1 Hz), 2.45 (3 H, s), 2.21 (1 H, t, *J* 2.6 Hz), 1.26 (6 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 164.0 (C), 135.4 (C), 132.2 (C), 130.1 (C), 127.2 (C), 125.3

(CH), 121.3 (CH), 109.8 (CH), 103.0 (CH), 78.9 (C), 72.1 (CH), 43.5 (b, CH_2), 39.8 (b, CH_2), 33.6 (CH_2), 21.5 (CH_2), 14.0 (b, CH_3); *m*/*z* (+ESI) 291 ($[M+Na]^+$, 9), 270 (19), 269 ($[M+H]^+$, 100).

1-((2*H*-1,2,3-Triazol-4-yl)methyl)-*N*,*N*-diethyl-5-methyl-1*H*-indole-2-carboxamide (13)

To a suspension of **38** (203 mg, 0.756 mmol, 1.00 equiv.) in *tert*-butanol (2 mL) and water (2 mL) was added azidotrimethylsilane (996 μL, 7.57 mmol, 10.0 equiv.), tetra-*n*-butylammonium fluoride (1 M in THF, 757 μL, 1.00 equiv.), sodium ascorbate (45 mg, 0.23 mmol, 0.30 equiv.) and copper(II) sulfate pentahydrate (19 mg, 0.076 mmol, 10 mol%). The reaction mixture was heated in a microwave reactor at 80 °C and 100 W for 3 h, cooled to room temperature and diluted with water (5 mL). The mixture was extracted with ethyl acetate (3×10 mL) and the combined organic layers were washed with water (30 mL), brine (30 mL) and dried (anhydrous $MgSO_4$). The solvent was removed *in vacuo* and the crude product was purified by column chromatography (40–60% ethyl acetate in hexane) and recrystallisation (diisopropyl ether) to give **13** (191 mg, 0.613 mmol, 81%) as colorless crystals; mp 110.6–112.8 °C (from dichloromethane); R_f 0.07 (50% ethyl acetate in hexane); (Found: C, 65.5; H, 6.8; N, 22.5. $C_{17}H_{21}N_5O$ requires C, 65.6; H, 6.8; N, 22.5%); (Found (+ESI): $[M+Na]^+$, 334.1639. $C_{17}H_{21}N_5O$+Na requires 334.1638); ν_{max} (neat)/cm^{-1} 3139 (b, NH), 2923, 2871, 1601 (CO), 1534, 1455, 1272, 1164, 974, 794; δ_H (300 MHz; $CDCl_3$) 10.3 (1 H, bs), 7.70 (1 H, bs), 7.39 (1 H, s), 7.31 (1 H, d, *J* 8.5 Hz), 7.09 (1 H, dd, *J* 8.5, 1.7 Hz), 6.58 (1 H, s), 5.46 (2 H, s), 3.57 (4 H, q, *J* 6.9 Hz), 2.42 (3 H, s), 1.24 (6 H, t, *J* 7.0 Hz); δ_C (125 MHz; $CDCl_3$) 164.4 (C), 135.5 (C), 131.7 (C), 130.8 (b, CH), 130.3 (C), 127.0 (C), 125.7 (CH), 121.3 (CH), 109.8 (CH), 103.1 (CH), one unresolved aryl peak (C), 43.8 (CH_2), 40.1 (CH_2), 38.9 (CH_2), 20.2 (CH_3), 14.6 (CH_3), 12.9 (CH_3); *m*/*z* (−ESI) 621 ($[2M-H]^-$, 61%), 310 ($[M-H]^-$, 100).

Ethyl 1-(4-Methoxybenzyl)-5-nitro-1*H*-indole-2-carboxylate (40)

General Procedure B was followed with ethyl 5-nitro-1*H*-indole-2-carboxylate (1.499 g, 6.400 mmol, 1.00 equiv.) and 4-methoxybenzyl chloride (971 μL, 7.16 mmol, 1.12 equiv.). Column chromatography (25–50% dichloromethane in hexane) gave **40** (1.248 g, 3.522 mmol, 55%) as a colorless solid; mp 131.1–133.4 °C (from dichloromethane); R_f 0.21 (20% ethyl acetate in

hexane); (Found: C, 64.3; H, 5.1; N, 7.9. $C_{19}H_{18}N_2O_5$ requires C, 64.4; H, 5.1; N, 7.9%); ν_{max} (neat)/cm^{-1} 2915, 2849, 1713 (CO), 1613, 1514, 1335, 1247, 1188, 1097, 747; δ_H (300 MHz; $CDCl_3$) 8.63 (1 H, d, *J* 2.0 Hz), 8.15 (1 H, dd, *J* 9.2, 2.1 Hz), 7.50 (1 H, s), 7.42 (1 H, d, *J* 9.2 Hz), 7.02 (2 H, d, *J* 8.7 Hz), 6.79 (2 H, d, *J* 8.7 Hz), 5.80 (2 H, s), 4.38 (2 H, q, *J* 7.1 Hz), 3.73 (3 H, s), 1.40 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 161.3 (C), 159.2 (C), 142.7 (C), 141.7 (C), 131.1 (C), 129.2 (C), 127.8 (CH), 125.3 (C), 120.2 (CH), 120.0 (CH), 114.3 (CH), 112.9 (CH), 111.3 (CH), 61.4 (CH_2), 55.3 (CH_3), 48.0 (CH_2), 14.4 (CH_3); *m*/*z* (+APCI) 341 (25%), 339 ($[M-CH_3]^+$, 31), 325 ($[M-(CH_2CH_3)]^+$, 100), 219 ($[M-(C_6H_4OCH_3)-(CH_2CH_3)+H]^+$, 18).

Ethyl 5-Amino-1-(4-methoxybenzyl)-1*H*-indole-2-carboxylate (41)

Palladium on carbon (75 mg, 10% w/w) was added to a suspension of **40** (738 mg, 2.08 mmol, 1.00 equiv.) in methanol (70 mL) and the reaction mixture was stirred under a balloon of hydrogen for 3 h. The reaction mixture was filtered through a pad of Celite® and the solvent was removed *in vacuo* to give **41** (665 mg, 2.05 mmol, 99%) as a brown gummy residue; R_f 0.51 (50% ethyl acetate in hexane); (Found (+ESI): $[M+Na]^+$, 347.1365. $C_{19}H_{20}N_2O_3$+Na requires 347.1366); ν_{max} (neat)/cm^{-1} 3432 (b, NH), 3362 (b, NH), 2932, 2835, 1698 (CO), 1512, 1459, 1245, 1218, 1176, 1091, 1028, 762; δ_H (300 MHz; $CDCl_3$) 7.18 (1 H, d, *J* 8.1 Hz), 7.17 (1 H, s), 7.00 (1 H, d, *J* 8.4 Hz), 6.94 (1 H, s, *J* 1.8 Hz), 6.80–6.76 (3 H, m), 5.71 (2 H, s), 4.32 (2 H, q, *J* 7.1 Hz), 3.74 (3 H, s), 3.54 (2 H, bs), 1.36 (3 H, t, *J* 7.2 Hz); δ_C (75 MHz; $CDCl_3$) 162.2 (C), 158.8 (C), 140.4 (C), 134.9 (C), 130.8 (C), 127.9 (C), 127.7 (CH), 127.1 (C), 117.1 (CH), 114.0 (CH), 111.7 (CH), 109.6 (CH), 106.0 (CH), 60.6 (CH_2), 55.3 (CH_3), 47.5 (CH_2), 14.5 (CH_3); *m*/*z* (+ESI) 671 ($[2M+Na]^+$, 51%), 649 ($[2M+H]^+$, 75), 648 ($[2M]^+$, 100), 347 ($[M+Na]^+$, 9), 325 ($[M+H]^+$, 83). The compound was used without further purification.

General Procedure F: Sulfonylation/Acetylation of Amines

Acetyl chloride (1.09 equiv.) or methanesulfonyl chloride (1.04 equiv.) was added dropwise to a stirring solution of amine (1.00 equiv.) and triethylamine (1.20 equiv.) in dichloromethane (3 mL/mmol of amine) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h before warming to room temperature and stirring for an additional 1–2 h. The reaction mixture was diluted

with dichloromethane and washed with water. The aqueous layer was re-extracted twice with dichloromethane and the combined organic layers were washed with saturated sodium bicarbonate solution, aqueous hydrochloric acid (1 M) and brine and dried over anhydrous $MgSO_4$. Solvents were removed *in vacuo* and the crude product was purified by column chromatography.

Ethyl 1-(4-Methoxybenzyl)-5-(methylsulfonamido)-1*H*-indole-2-carboxylate (42)

General Procedure F was followed with **41** (186 mg, 0.573 mmol, 1.00 equiv.) and methanesulfonyl chloride (46 μL, 0.594, 1.04 equiv.) Column chromatography (20–40% ethyl acetate in hexane) gave **42** (191 mg, 0.475 mmol, 83%) as a purple gummy mass; R_f 0.38 (50% ethyl acetate in hexane); (Found (+ESI): $[M+Na]^+$, 425.1140. $C_{20}H_{22}N_2O_5S$+Na requires 425.1142); ν_{max} (neat)/cm^{-1} 3260 (b, NH), 2933, 2837, 1705 (CO), 1513, 1462, 1322, 1246, 1151, 1094, 1027, 971, 762; δ_H (300 MHz; $CDCl_3$) 7.61 (1 H, d, *J* 2.1 Hz), 7.36 (1 H, d, *J* 9.0 Hz), 7.33 (1 H, s), 7.20 (1 H, dd, *J* 8.9, 2.0 Hz), 7.02 (2 H, d, *J* 8.7 Hz), 6.79 (2 H, d, *J* 8.4 Hz), 6.61 (1 H, bs), 5.76 (2 H, s), 4.35 (2 H, q, *J* 7.1 Hz), 3.74 (3 H, s), 2.97 (3 H, s), 1.37 (3 H, t, *J* 7.2 Hz); δ_C (75 MHz; $CDCl_3$) 161.9 (C), 159.0 (C), 137.9 (C), 130.1 (C), 129.8 (C), 129.2 (C), 127.9 (CH), 126.6 (C), 121.9 (CH), 116.9 (CH), 114.2 (CH), 112.2 (CH), 110.8 (CH), 61.0 (CH_2), 55.4 (CH_3), 47.7 (CH_2), 39.1 (CH_3), 14.4 (CH_3); *m/z* (−ESI) 803 ($[2M-H]^-$, 13%), 401 ($[M-H]^-$, 100).

Ethyl 5-Acetamido-1-(4-methoxybenzyl)-1*H*-indole-2-carboxylate (43)

General Procedure F was followed with **41** (275 mg, 0.848 mmol, 1.00 equiv.) and acetyl chloride (66 μL, 0.928 mmol, 1.09 equiv.). Column chromatography (25–40% ethyl acetate in hexane) gave **43** (279 mg, 0.761 mmol, 90%) as a tan solid; mp 156.0–157.2 °C (from dichloromethane); R_f 0.30 (50% ethyl acetate in hexane); (Found: C, 68.8; H, 6.0; N, 7.6. $C_{21}H_{22}N_2O_4$ requires C, 68.8; H, 6.1; N, 7.7%); (Found (+APCI): $[M+H]^+$, 367.1653. $C_{21}H_{22}N_3O_4$+H requires 367.1652); ν_{max} (neat)/cm^{-1} 3291 (b, NH), 2914, 2849, 1706 (CO), 1661 (CO), 1513, 1468, 1246, 1204, 1176, 1150, 1093, 1029, 764; δ_H (300 MHz; $CDCl_3$) 7.91 (1 H, s), 7.41 (1 H, bs), 7.30–7.26 (3 H, m), 6.98 (2 H, d, *J* 8.7 Hz), 7.76 (2 H, d, *J* 8.7 Hz), 5.73 (2 H, s), 4.33 (2 H, q, *J* 7.1 Hz), 3.73 (3 H, s), 2.17 (3 H, s), 1.36 (3 H, t, *J* 7.1 Hz); δ_C (75 MHz; $CDCl_3$) 168.5 (C), 162.0

(C), 158.9 (C), 137.0 (C), 131.4 (C), 130.4 (C), 128.6 (C), 127.7 (CH), 126.3 (C), 120.0 (CH), 114.1 (CH), 111.3 (2×CH), 111.0 (CH), 60.8 (CH_2), 55.4 (CH_3), 47.5 (CH_2), 24.5 (CH_3), 14.4 (CH_3); *m*/*z* (+ESI) 755 ($[2M+Na]^+$, 68%), 750 ($[2M+NH_4]^+$, 58), 733 ($[2M+H]^+$, 100), 640 (24), 389 ($[M+Na]^+$, 10), 384 ($[M+NH_4]^+$, 87), 367 ($[M+H]^+$, 42).

General procedure G: Alternate Procedure for Hydrolysis of Ethyl Esters

Sodium carbonate (5.00 equiv.) was added to a suspension of carboxylic acid (1.00 equiv.) in methanol (2.5 mL/mmol of ester) and water (1.25 mL/mmol of ester). The reaction mixture was stirred at room temperature for 1 week. Solvent was removed *in vacuo* and the resulting residue was dissolved in water, washed with ethyl acetate and acidified to pH 3 with aqueous hydrochloric acid (1 M). Filtration gave the desired carboxylic acids which were dried under high vacuum at 60 °C. Solvent was removed from the organic layer *in vacuo* to recover starting material.

1-(4-Methoxybenzyl)-5-(methylsulfonamido)-1*H*-indole-2-carboxylic Acid (44)

General Procedure G was followed with **42** (140 mg, 0.348 mmol) to give **44** (60 mg, 0.160 mmol, 46%) as a pale purple solid; mp 161.0–162.3 °C (from water); R_f 0.08 (5% methanol in dichloromethane); (Found (+ESI): $[M+Na]^+$, 397.0827. $C_{18}H_{18}N_2O_5S$+Na requires 397.0829); ν_{max} (neat)/cm^{-1} 3600–2200 (NH/COOH), 2931, 2838, 1692 (CO), 1513, 1461, 1318, 1245, 1176, 1146, 975, 764; δ_H (400 MHz; CD_3OD) 7.58 (1 H, d, *J* 2.0 Hz), 7.43 (1 H, d, *J* 9.0 Hz), 7.32 (1 H, s Hz), 7.21 (1 H, dd, *J* 8.9, 2.1 Hz), 7.01 (2 H, d, *J* 8.6 Hz), 6.78 (2 H, d, *J* 8.7 Hz), 5.79 (2 H, s), 3.71 (3 H, s), 2.89 (3 H, s); δ_C (100 MHz; CD_3OD) 164. 7 (C), 160.3 (C), 138.8 (C), 132.3 (C), 131.8 (C), 130.3 (C), 128.9 (CH), 127.8 (C), 122.6 (CH), 116.9 (CH), 114.8 (CH), 113.0 (CH), 111.8 (CH), 55.6 (CH_3), 48.1 (CH_2), 38.6 (CH_3); *m*/*z* (−ESI) 774 (49%), 748 (24), 747 ($[2M-H]^-$, 52), 374 (24), 373 ($[M-H]^-$, 100). Starting material (69 mg, 0.17 mmol, 49%) was recovered as a purple gummy mass.

5-Acetamido-1-(4-methoxybenzyl)-1*H*-indole-2-carboxylic Acid (38a)

General Procedure G was followed with **43** (176 mg, 0.480 mmol) to give **45** (80 mg, 0.236 mmol, 49%) as a tan solid; mp 198.2–200.1 °C (from water); R_f 0.05 (5% methanol in

dichloromethane); (Found (+ESI): $[M+Na]^+$, 361.1158. $C_{19}H_{18}N_2O_4$+Na requires 361.1159); ν_{max} (neat)/cm^{-1} 3700–2200 (COOH), 2914, 2849, 1695 (CO), 1666 (CO), 1512, 1457, 1243, 1201, 1175, 1031, 807; δ_H (400 MHz; $(CD_3)_2CO$) 8.15 (1 H, d, *J* 1.5 Hz), 7.46 (1 H, d, *J* 9.0 Hz), 7.42 (1 H, dd, *J* 9.0, 1.9 Hz), 7.34 (1 H, s), 7.07 (2 H, d, *J* 8.7 Hz), 6.80 (2 H, d, *J* 8.7 Hz), 5.83 (2 H, s), 3.71 (3 H, s), 2.07 (3 H, s); δ_C (100 MHz; $(CD_3)_2CO$) 167.5 (C), 162.4 (C), 158.8 (C), 136.2 (C), 133.1 (C), 130.6 (C), 127.9 (C), 127.8 (CH), 126.0 (C), 118.7 (CH), 113.6 (CH), 111.7 (CH), 111.0 (CH), 110.6 (CH), 54.4 (CH_3), 46.5 (CH_2), 23.1 (CH_3); *m*/*z* (−ESI) 338 (16%), 337 ($[M-H]^-$, 100), 212 (14), 159 (15). Starting material (86 mg, 0.23 mmol, 49%) was recovered as a tan solid. The recovered starting material was re-subjected to General Procedure G, with lithium hydroxide (8.4 mg, 0.35 mmol, 1.5 equiv.) instead of sodium carbonate, to give additional **45** (60 mg, 0.18 mmol, 75%) as a tan solid.

General Procedure H: Mild Amide Coupling Using PyBOP®

To a solution of carboxylic acid (1.0 equiv.) in dichloromethane (2.5 mL/mmol of acid) at 0 °C was added PyBOP® (1.0 equiv.), *N*,*N*-diisopropylethylamine (2.00 equiv.) and *N*-methylpropargylamine (1.4–2.0 equiv.). The reaction mixture was warmed to room temperature and stirred for 1.5–24 h until complete by TLC. Workup and purification according to General Procedure E gave the desired amides.

5-Acetamido-1-(4-methoxybenzyl)-*N*-methyl-*N*-(prop-2-yn-1-yl)-1*H*-indole-2-carboxamide (14)

General Procedure H was followed with **45** (104 mg, 0.307 mmol, 1.00 equiv.) and *N*-methylpropargylamine (52 μL, 0.62 mmol, 2.0 equiv.). Column chromatography (70–75% ethyl acetate in hexane) gave **14** (112 mg, 0.288 mmol, 94%) as a colorless foam; mp 75.4–78.9 °C (from dichloromethane); R_f 0.62 (10% methanol in dichloromethane); (Found: C, 70.5; H, 6.2; N, 10.5. $C_{23}H_{23}N_3O_3$ requires C, 70.9; H, 6.0; N, 10.8%); (Found (+ESI): $[M+Na]^+$, 412.1629. $C_{23}H_{23}N_3O_3$+Na requires 412.1632); ν_{max} (neat)/cm^{-1} 3289 (b, NH/propargyl CH), 2926, 2838, 1614 (CO), 1590, 1512, 1463, 1244, 1177, 1032, 846; δ_H (300 MHz; $CDCl_3$) 7.84 (1 H, s), 7.67 (1 H, bs), 7.23 (1 H, d, *J* 8.7 Hz), 7.13 (1 H, dd, *J* 8.7, 1.7 Hz), 6.98 (2 H, d, *J* 8.6 Hz),

6.73 (3 H, d and overlapping bs, *J* 8.7 Hz), 5.37 (2 H, s), 4.12 (2 H, bs), 3.72 (3 H, s), 3.04 (3 H, bs), 2.30 (1 H, bs), 2.16 (3 H, s); δ_C (75 MHz; $CDCl_3$) 168.8 (C), 164.4 (C), 159.0 (C), 135.2 (C), 131.7 (C), 131.1 (C), 130.2 (C), 128.4 (CH), 126.7 (C), 118.4 (CH), 114.1 (CH), 113.8 (CH), 110.6 (CH), 105.1 (CH), 78.4 (C), 72.9 (CH), 55.4 (CH_3), 47.3 (CH_2), 24.4 (CH_3); *m/z* (+ESI) 801 ($[2M+Na]^+$, 50%), 796 ($[2M+NH_4]^+$, 47), 779 ($[2M+H]^+$, 77), 588 (100), 407 ($[M+NH_4]^+$, 59), 390 ($[M+H]^+$, 52).

1-(4-Methoxybenzyl)-*N*-methyl-5-(methylsulfonamido)-*N*-(prop-2-yn-1-yl)-1*H*-indole-2-carboxamide (15)

General Procedure H was followed with **44** (50 mg, 0.13 mmol, 1.00 equiv.) and *N*-methylpropargylamine (16 μL, 0.19 mmol, 1.4 equiv.). Column chromatography (30–45% ethyl acetate in hexane) gave **15** (48 mg, 0.11 mmol, 85%) as a pale pink foam; mp 78.1–80.6 °C (from dichloromethane); R_f 0.26 (50% ethyl acetate in hexane); (Found (+ESI): $[M+Na]^+$, 448.1302. $C_{22}H_{23}N_3O_4S$+Na requires 448.1302); ν_{max} (neat)/cm^{-1} 3268 (b, NH/propargyl CH), 2927, 2839, 1613 (CO), 1513, 1460, 1323, 1245, 1150, 973, 735; δ_H (400 MHz; $CDCl_3$) 7.55 (1 H, d, *J* 1.1 Hz), 7.34 (1 H, d, *J* 8.8 Hz), 7.12 (1 H, dd, *J* 8.8, 2.1 Hz), 7.03 (2 H, d, *J* 8.2 Hz), 6.82 (1 H, bs), 6.76 (2 H, d, *J* 8.8 Hz), 6.64 (1 H, s), 5.40 (2 H, s), 4.30 (1 H, bs), 4.00 (1 H, bs), 3.74 (3 H, s), 3.06 (3 H, bs), 2.96 (3 H, s), 2.33 (1 H, bs); δ_C (100 MHz; $CDCl_3$) 164.1 (C), 159.1 (C), 136.2 (C), 132.5 (C), 129.9 (C), 129.3 (C), 128.5 (CH), 127.1 (C), 120.4 (CH), 116.7 (CH), 114.1 (CH), 111.4 (CH), 104.7 (CH), 78.3 (C), 73.5 (b, CH), 72.6 (b, CH), 55.4 (CH_3), 47.5 (CH_2), 41.5 (b, CH_2), 39.0 (CH_3), 36.5 (b, CH_2), 32.8 (b, CH_3); *m/z* (−ESI) 849 ($[2M-H]^-$, 18%), 613 (10), 425 (27), 424 ($[M-H]^-$, 100), 357 (10).

Ethyl 1-((6-Methoxypyridin-3-yl)methyl)-5-methyl-1*H*-indole-2-carboxylate (47)

General Procedure B was followed with ethyl 5-methyl-1*H*-indole-2-carboxylate (264 mg, 1.30 mmol, 1.00 equiv.) and 5-(chloromethyl)-2-methoxypyridine (229 mg, 1.45 mmol, 1.12 equiv.). Column chromatography (20% ethyl acetate in hexane) gave **47** (404 mg, 1.25 mmol, 96%) as a colorless solid; mp 98.5–98.9 °C (from dichloromethane); R_f 0.57 (30% ethyl acetate in hexane); (Found: C, 70.1; H, 6.1; N, 8.5. $C_{19}H_{20}N_2O_3$ requires C, 70.4; H, 6.2; N, 8.6%); ν_{max}

(neat)/cm^{-1} 2943, 1714 (CO), 1609, 1491, 1243, 1194, 1127, 1089, 1023, 792, 762; δ_H (400 MHz; $CDCl_3$) 8.01 (1 H, d, *J* 2.0 Hz), 7.46 (1 H, s), 7.32 (1 H, dd, *J* 8.6, 2.4 Hz), 7.29–7.27 (2 H, m), 7.15 (1 H, dd, *J* 8.7, 1.3 Hz), 6.61 (1 H, d, *J* 8.6 Hz), 5.73 (2 H, s), 4.34 (2 H, q, *J* 7.1), 3.88 (3 H, s), 2.43 (3 H, s), 1.38 (3 H, t, *J* 7.1 Hz); δ_C (100 MHz; $CDCl_3$) 163.7 (C), 162.2 (C), 145.3 (CH), 137.9 (C), 137.7 (CH), 130.4 (C), 127.50 (CH), 127.49 (C), 126.8 (C), 126.6 (C), 122.2 (CH), 111.0 (CH), 110.9 (CH), 110.4 (CH), 60.7 (CH_2), 53.5 (CH_3), 45.1 (CH_2), 21.4 (CH_3), 14.5 (CH_3); *m*/*z* (+ESI) 326 (27%), 325 ([M+H]$^+$, 100).

1-((6-Methoxypyridin-3-yl)methyl)-5-methyl-1*H*-indole-2-carboxylic Acid (48)

Sodium hydroxide (25 mg, 0.63 mmol, 1.01 equiv.) was added to a suspension of **47** (200 mg, 0.617 mmol, 1.00 equiv.) in ethanol (2.5 mL) and water (1.25 mL). The reaction mixture was heated under reflux for 4 h. Solvent was removed *in vacuo* and the resulting sodium salt was dissolved in a minimal volume of water and acidified to pH 4. Filtration gave **48** (166 mg, 0.560 mmol, 91%) as a colorless powder; mp 211.7–212.6 °C (from water); R_f 0.13 (5% methanol in dichloromethane); (Found: C, 68.8; H, 5.5; N, 9.4. $C_{17}H_{16}N_2O_3$ requires C, 68.9 H, 5.4; N, 9.5%); ν_{max} (neat)/cm^{-1} 3500–2100 (OH), 3012, 2950, 1697 (CO), 1494, 1262, 1250, 1198, 1124, 1031, 1021, 793; δ_H (300 MHz; $(CD_3)_2SO$) 13.0 (1 H, bs), 8.01 (1 H, d, *J* 2.1 Hz), 7.54 (1 H, d, *J* 8.4 Hz), 7.45 (1 H, s), 7.37 (1 H, dd, *J* 8.4, 2.4 Hz), 7.21 (1 H, s), 7.14 (1 H, dd, *J* 8.6, 1.2 Hz), 6.70 (1 H, d, *J* 8.7), 5.77 (2 H, s), 3.77 (3 H, s), 2.36 (3 H, s); δ_C (75 MHz; $(CD_3)_2SO$) 163.0 (C), 162.8 (C), 145.2 (CH), 137.8 (CH), 137.3 (C), 129.5 (C), 127.8 (C), 127.2 (C), 126.9 (CH), 125.9 (C), 121.6 (CH), 111.0 (CH), 110.4 (CH), 110.2 (CH), 53.1 (CH_3), 44.0 (CH_2), 20.9 (CH_3); *m*/*z* (−ESI) 591 ([2M−H]$^-$, 24%), 295 ([M−H]$^-$, 100).

1-((6-Methoxypyridin-3-yl)methyl)-*N*,5-dimethyl-*N*-(prop-2-yn-1-yl)-1*H*-indole-2-carboxamide (16)

General Procedure H was followed with **48** (145 mg, 0.489 mmol, 1.00 equiv.) and *N*-methylpropargylamine (62 μL, 0.73 mmol, 1.5 equiv.). Column chromatography (30–40% ethyl acetate in hexane) gave **16** (161 mg, 0.463 mmol, 95%) as a colorless gummy mass; R_f 0.54 (50% ethyl acetate in hexane); (Found (+ESI): [M+Na]$^+$, 370.1526. $C_{21}H_{21}N_3O_2$+Na

requires 370.1526); ν_{max} (neat)/cm^{-1} 3287 (propargyl CH), 2945, 2919, 1627 (CO), 1609, 1491, 1393, 1285, 1261, 1128, 1024, 793; δ_H (300 MHz; $CDCl_3$) 8.01 (1 H, d, *J* 2.1 Hz), 7.43 (1 H, s), 7.37 (1 H, dd, *J* 8.7, 2.4 Hz), 7.26 (1 H, d, *J* 8.4 Hz), 7.10 (1 H, dd, *J* 8.4, 0.9 Hz), 6.75 (1 H, bs), 6.59 (1 H, d, *J* 8.4 Hz), 5.42 (2 H, s), 4.25 (2 H, bs), 3.87 (3 H, s), 3.14 (3 H, s), 2.43 (3 H, s), 2.32 (1 H, bs); δ_C (75 MHz; $CDCl_3$) 164.3 (C), 163.7 (C), 145.5 (CH), 138.1 (CH), 136.2 (C), 130.7 (C), 130.1 (C), 126.9 (C), 126.7 (C), 125.9 (CH), 121.5 (CH), 111.0 (CH), 110.1 (CH), 105.1 (CH), 78.5 (C), 72.9 (CH), 53.5 (CH_3), 45.1 (CH_2), 21.5 (CH_3); *m/z* (+ESI) 717 ([2M+Na]$^+$, 49%), 695 ([2M+H]$^+$, 100), 370 ([M+Na]$^+$, 7), 348 ([M+H]$^+$, 57).

General procedure I: Reductive alkylation of 3,5-dimethylaniline. A solution of 3,5-dimethylaniline (1.25 mL, 10 mmol) and the appropriately substituted benzaldehyde (10 mmol, 1.0 equiv.) in 1,2-dichloroethane (35 mL) was stirred at ambient temperature for 0.5 h, and then treated with $NaBH(OAc)_3$ (3.18 g, 15 mmol, 1.5 equiv.) in a single portion. After stirring for 6 h, the mixture was quenched by the addition of 1 M aq. NaOH solution (65 mL), and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic phases were washed with brine (100 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The crude products were purified by recrystallization (solids) or flash chromatography (oils).

_N_-benzyl-3,5-dimethylaniline (55)

Subjecting benzaldehyde (1.00 mL, 10 mmol) to the general procedure I afforded, following purification by flash chromatography (hexane-EtOAc, 95:5), **55** (1.77 g, 84%) as a pale yellow oil. R_f 0.50 (hexane-EtOAc, 90:10); ν_{max} (neat)/cm^{-1} 3410, 3027, 2915, 2855, 1599, 1451, 1335, 1240, 1180, 1028, 819, 732, 690; δ_H (400 MHz, $CDCl_3$) *δ* 7.39-7.34 (4H, m), 7.30-7.27 (1H, m), 6.42 (1H, s), 6.32 (2H, s), 4.32 (2H, s), 4.15 (1H, br s), 2.25 (6H, s); δ_C (100 MHz, $CDCl_3$) *δ* 148.3 (C), 139.7 (C), 139.1 (C), 128.7 (CH), 127.8 (CH), 127.3 (CH), 120.0 (CH), 111.1 (CH), 48.7 (CH_2), 21.6 (CH_3); *m/z* (+HRESI) 212.1433 ([*M* + H]$^+$, calcd for $C_{15}H_{18}N$: 212.1434).

_N_-(4-methoxybenzyl)-3,5-dimethylaniline (56)

Subjecting 4-methoxybenzaldehyde (1.22 mL, 10 mmol) to the to the general procedure I afforded, following recrystallization from *i*-PrOH, **56** (1.91 g, 79%) as a colorless crystalline solid. R_f 0.63 (hexane-EtOAc, 80:20); m.p. 48–50 °C; ν_{max} (neat)/cm^{-1} 3407, 2912, 2834, 1599, 1509, 1464, 1335, 1301, 1244, 1173, 1033, 820, 690; δ_H (400 MHz, $CDCl_3$) δ 7.29 (2H, d, J = 8.6 Hz, ArH), 6.88 (2H, d, J 8.6 Hz), 6.41 (1H, s), 6.31 (2H, s), 4.23 (2H, s), 3.81 (3H, s), 2.24 (6H, s); δ_C (100 MHz, $CDCl_3$) δ 159.0 (C), 148.2 (C), 139.1 (C), 131.6 (C), 129.1 (CH), 120.0 (CH), 114.2 (CH), 111.2 (CH), 55.4 (CH_3), 48.2 (CH_2), 21.6 (CH_3); *m*/*z* (+HRESI) 254.1354 ([M + Na]$^+$, calcd for $C_{16}H_{19}NNaO$: 264.1359).

_N_-(4-(2-fluoroethoxy)benzyl)-3,5-dimethylaniline (57)

Subjecting 4-(2-fluoroethoxy)benzaldehyde (1.68 g, 10 mmol) to the to the general procedure I afforded, following purification by flash chromatography (hexane-EtOAc, 90:10), **57** (1.90 g, 69%) as a white solid. R_f 0.28 (hexane-EtOAc, 90:10); m.p. 55–57 °C; ν_{max} (neat)/cm^{-1} 3410, 2952, 2916, 2862, 1599, 1509, 1336, 1245, 1176, 1053, 922, 821, 691; δ_H (500 MHz, $CDCl_3$) δ 7.29 (2H, d, J 8.7 Hz,), 6.91 (2H, d, J = 8.7 Hz), 6.39 (1H, s), 6.29 (2H, s), 4.76 (2H, dt, $^2J_{HF}$ 47.4 Hz, $^3J_{HH}$ 4.2 Hz), 4.24 (2H, s), 4.21 (2H, dt, $^3J_{HF}$ 27.8 Hz, $^3J_{HH}$ 4.2 Hz), 2.24 (6H, s); δ_C (125 MHz, $CDCl_3$) δ 157.8 (C), 148.4 (C), 139.1 (C), 132.5 (C), 129.0 (CH), 119.8 (CH), 114.9 (CH), 111.0 (CH), 82.1 (d, $^1J_{CF}$ 170.7 Hz), 67.3 (d, $^2J_{CF}$ 20.6 Hz), 48.0 (CH_2), 21.6 (CH_3); δ_F (282 MHz, $CDCl_3$) δ –223.78 (m, 1F); *m*/*z* (+HRESI) 296.1423 ([M + Na]$^+$, calcd for $C_{17}H_{20}FNNaO$: 296.1421).

_N_-(4-isopropoxybenzyl)-3,5-dimethylaniline (58)

Subjecting 4-isopropoxybenzaldehyde (1.64 g, 10 mmol) to the to the general procedure I afforded, following purification by flash chromatography (hexane-EtOAc, 90:10), **58** (2.27 g, 84%) as a white solid. R_f 0.60 (hexane-EtOAc, 80:20); m.p. 53–54 °C; ν_{max} (neat)/cm^{-1} 3408, 2975, 2916, 1499, 1507, 1334, 1237, 1180, 1116, 953, 818, 689; δ_H (500 MHz, $CDCl_3$) δ 7.28 (2H, d, J 8.5 Hz), 6.88 (2H, d, J 8.5 Hz), 6.41 (1H, s), 6.31 (2H, s), 4.55 (1H, sep, J 6.0 Hz), 4.23 (2H, s), 3.98 (1H, br s), 2.25 (6H, s), 1.35 (6H, d, J 6.0 Hz); δ_C (125 MHz, $CDCl_3$) δ 157.3 (C), 148.4 (C), 139.0 (C), 131.5 (C), 129.1 (CH), 119.8 (CH), 116.1 (CH), 111.0 (CH), 70.1 (CH), 48.1

(CH_2), 22.2 (CH_3), 21.6 (CH_3); *m/z* (+HRESI) 270.1853 ([*M* + H]$^+$, calcd for $C_{18}H_{24}NO$: 270.1852).

***N*-(4-(benzyloxy)benzyl)-3,5-dimethylaniline (59)**

Subjecting 4-(benzyloxy)benzaldehyde (2.12 g, 10 mmol) to the to the general procedure I afforded, following purification by flash chromatography (hexane-EtOAc, 90:10), **59** (3.04 g, 96%) as a pale orange oil. R_f 0.26 (hexane-EtOAc, 80:20); ν_{max} (neat)/cm^{-1} 3408, 3030, 2913, 2860, 1599, 1508, 1454, 1335, 1238, 1172, 1024, 820, 735, 693; δ_H (500 MHz, $CDCl_3$) *δ* 7.45-7.43 (2H, m), 7.41-7.38 (2H, m), 7.35-7.32 (1H, m), 7.29 (2H, d, *J* 8.7 Hz), 6.39 (1H, s), 6.29 (2H, s), 5.07 (2H, s), 4.23 (2H, s), 3.83 (1H, br s), 2.24 (6H, s); δ_C (125 MHz, $CDCl_3$) *δ* 158.2 (C), 148.5 (C), 139.1 (C), 137.2 (C), 132.1 (C), 129.0 (CH), 128.7 (CH), 128.1 (CH), 127.6 (CH), 119.7 (CH), 115.1 (CH), 110.9 (CH), 70.2 (CH_2), 48.0 (CH_2), 21.6 (CH_3); *m/z* (+HRESI) 340.1673 ([*M* + Na]$^+$, calcd for $C_{22}H_{23}NNaO$: 340.1672).

General procedure J: Acylation of *N*-substituted anilines. To a solution of the appropriate aniline (2 mmol) in glacial acetic acid (10 mL) was added acetic anhydride (380 µL, 4 mmol, 2 equiv.) and the solution stirred at ambient temperature for 1.5 h. The reaction was quenched by pouring onto 1 M aq. sodium hydroxide (200 mL), and the aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The obtained crude products were purified by flash chromatography on silica.

***N*-(3,5-Dimethylphenyl)-*N*-(benzyl)acetamide (17)**

Subjecting **55** (505 mg, 2.39 mmol) to the general procedure J afforded, following purification by flash chromatography (hexane-EtOAc, 80:20), gave **17** (502 mg, 83%) as a colorless oil. R_f 0.17 (hexane-EtOAc, 80:20); (Found: C 79.96, H 7.76, N 5.46; $C_{17}H_{19}NO$ requires C 80.60, H

7.56, N 5.53); ν_{max} (neat)/cm^{-1} 3030, 2919, 1655, 1594, 1387, 1304, 1243, 976, 853, 709; δ_H (300 MHz, $CDCl_3$) *δ* 7.22 (5H, m), 6.90 (1H, s), 6.59 (2H, s), 4.85 (2H, s), 2.23 (6H, s), 1.89 (3H, s); δ_C (75 MHz, $CDCl_3$) *δ* 170.4 (C), 142.8 (C), 139.1 (C), 137.7 (C), 129.4 (CH), 128.7 (CH), 128.2 (CH), 127.2 (CH), 125.7 (CH), 52.8 (CH_2), 22.6 (CH_3), 21.1 (CH_3); *m*/*z* (+ESI) 529 ([2*M* + Na]$^+$, 80%), 276 ([*M* + Na]$^+$, 100).

_N_-(3,5-Dimethylphenyl)-_N_-(4-methoxybenzyl)acetamide (18)

Subjecting **56** (483 mg, 2.00 mmol) to the general procedure J afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **18** (429 mg, 76%) as a colorless oil that crystallized on prolonged standing. R_f 0.17 (hexane-EtOAc, 80:20); m.p. 68–70 °C; (Found: C 76.17, H 7.75, N 4.94, $C_{18}H_{21}NO_2$ requires C 76.30, H 7.47, N 4.94); ν_{max} (neat)/cm^{-1} 2955, 2929, 2918, 2832, 1641, 1589, 1512, 1429, 1379, 1306, 1241, 1177, 1028, 913, 860, 812, 717, 664; δ_H (500 MHz, $CDCl_3$) *δ* 7.12 (2H, d, *J* 8.6 Hz), 6.92 (1H, s), 6.79 (2H, d, *J* 8.6 Hz), 6.57 (2H, s), 4.77 (2H, s), 3.78, (3H, s), 2.25 (6H, s), 1.86 (3H, s); δ_C (125 MHz, $CDCl_3$) *δ* 170.5 (C), 158.9 (C), 143.0 (C), 139.3 (C), 130.3 (CH), 130.2 (C), 129.5 (CH), 125.9 (CH), 113.7 (CH), 55.4 (CH_3), 52.3 (CH_2), 22.9 (CH_3), 21.2 (CH_3); *m*/*z* (+ESI) 306 ([*M* + Na]$^+$, 100%).

_N_-(3,5-Dimethylphenyl)-_N_-(4-(2-fluoroethoxy)benzyl)acetamide (19)

Subjecting **57** (547 mg, 2.00 mmol) to the general procedure J afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **19** (617 mg, 98%) as a colorless oil that crystallized on prolonged standing. R_f 0.19 (hexane-EtOAc, 80:20); m.p. 76–78 °C; (Found: C 72.14, H 7.11, N 4.47; $C_{19}H_{22}FNO_2$ requires C 72.36, H 7.03, N 4.44); ν_{max} (neat)/cm^{-1} 2960, 2927, 1637, 1593, 1509, 1432, 1396, 1305, 1247, 1174, 1112, 1074, 1046, 974, 922, 876, 854, 806, 765, 705, 659; δ_H (500 MHz, $CDCl_3$) *δ* 7.12 (2H, d, *J* 8.6 Hz), 6.92 (1H, s), 6.81 (2H, d, *J* 8.6 Hz), 6.57 (2H, s), 4.78 (2H, s), 4.73 (2H, dt, $^2J_{HF}$ 47.3 Hz, $^3J_{HH}$ 4.2 Hz), 4.18 (2H, dt, $^3J_{HF}$ 27.8 Hz, $^3J_{HH}$ 4.2 Hz), 2.25 (6H, s), 1.86 (3H, s); δ_C (125 MHz, $CDCl_3$) *δ* 170.5 (C), 157.8 (C), 142.9 (C), 139.3 (C), 130.8 (C), 130.3 (CH), 129.6 (CH), 125.9 (CH), 114.5 (CH), 82.1 (d, $^1J_{CF}$ = 170.6 Hz), 67.2 (d, $^2J_{CF}$ = 20.5 Hz), 52.3 (CH_2), 22.8 (CH_3), 21.3 (CH_3); δ_F (282 MHz, $CDCl_3$) *δ* –223.91 (m, 1F); *m*/*z* (+ESI) 338 ([*M* + Na]$^+$, 100%.

***N*-(3,5-Dimethylphenyl)-*N*-(4-isopropoxybenzyl)acetamide (20)**

Subjecting **58/5** (539 mg, 2.00 mmol) to the general procedure J afforded, following purification by flash chromatography (hexane-EtOAc, 80:20), gave **20** (512 mg, 82%) as a colorless oil. R_f 0.19 (hexane-EtOAc, 80:20); (Found: C 76.90, H 8.11, N 4.39; $C_{20}H_{25}NO_2$ requires C 77.14, H 8.09, N 4.50); ν_{max} (neat)/cm^{-1} 2975, 2920, 1655, 1608, 1594, 1507, 1383, 1301, 1232, 1180, 1119, 953, 854, 707; δ_H (500 MHz, $CDCl_3$) δ 7.09 (2H, d, *J* 8.5 Hz), 6.91 (1H, s), 6.77 (2H, d, *J* 8.5 Hz), 6.57 (2H, s), 4.76 (2H, s), 4.51 (1H, sep, *J* 6.1 Hz), 2.25 (6H, s), 1.86 (3H, s), 1.31 (6H, d, *J* 6.1 Hz); δ_C (125 MHz, $CDCl_3$) δ 170.5 (C), 157.2 (C), 143.0 (C), 139.2 (C), 130.3 (CH), 130.0 (C), 129.5 (CH), 126.0 (CH), 115.8 (CH), 70.0 (CH), 52.3 (CH_2), 22.9 (CH_3), 22.2 (CH_3), 21.3 (CH_3); *m*/*z* (+ESI) 645 ([2*M* + Na]$^+$, 50%), 334 ([*M* + Na]$^+$, 100).

***N*-(3,5-Dimethylphenyl)-*N*-(4-(benzyloxy)benzyl)acetamide (21)**

Subjecting **59** (635 mg, 2.00 mmol) to the general procedure J afforded, following purification by flash chromatography (hexane-EtOAc, 80:20), gave **21** (706 mg, 98%) as a colorless solid. R_f 0.18 (hexane-EtOAc, 80:20); m.p. 81–84 °C; (Found: C 79.96, H 7.39, N 3.87; $C_{24}H_{25}NO_2$ requires C 80.19, H 7.01, N 3.90); ν_{max} (neat)/cm^{-1} 2938, 2913, 1637, 1584, 1509, 1399, 1307, 1234, 1171, 1111, 1013, 857, 842, 802, 747, 726, 700, 659, 620; δ_H (500 MHz, $CDCl_3$) δ 7.42 (2H, d, *J* 7.2 Hz), 7.38 (2H, t, *J* 7.2 Hz), 7.32 (1H, t, *J* 7.2 Hz), 7.12 (2H, d, *J* 8.5 Hz), 6.92 (1H, s), 6.88 (2H, d, *J* 8.5 Hz), 6.58 (2H, s), 5.04 (2H, s), 4.78 (2H, s), 2.25 (6H, s), 1.87 (3H, s); δ_C (125 MHz, $CDCl_3$) δ 170.5 (C), 158.1 (C), 143.0 (C), 139.3 (C), 137.2 (C), 130.4 (C), 130.3 (CH), 129.6 (CH), 128.7 (CH), 128.1 (CH), 127.6 (CH), 125.9 (CH), 114.7 (CH), 70.1 (CH_2), 52.3 (CH_2), 22.8 (CH_3), 21.3 (CH_3); *m*/*z* (+ESI) 741 ([2*M* + Na]$^+$, 5%), 382 ([*M* + Na]$^+$, 100).

General procedure K: Alkylation of *N*-substituted anilines with *N*,*N*-diethylchloroacetamide.
A solution of the appropriate *N*-substituted aniline (3 mmol) in acetonitrile (15 mL) was treated with *N*,*N*-diethylchloroacetamide (539 mg, 3.6 mmol, 1.2 equiv.), potassium carbonate (1 451 mg, 10.5 mmol, 3.5 equiv.) and potassium iodide (598 mg, 3.6 mmol, 1.2 equiv.) and the mixture stirred at 60 °C for 40 h. Solvent was evaporated under reduced

pressure, and the residue was partitioned between H_2O (200) and CH_2Cl_2 (50 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and the solvent evaporated under reduced pressure. The obtained crude products were purified by flash chromatography on silica.

2-((3,5-Dimethylphenyl)(benzyl)amino)-*N*,*N*-diethylacetamide (22)

Subjecting **17** (505 mg, 2.39 mmol) to the general procedure K afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **22** (652 mg, 84%) as a white solid. R_f 0.20 (hexane-EtOAc, 80:20); m.p. 89–91 °C; (Found: C 77.53, H 8.20, N 8.49; $C_{21}H_{28}N_2O$ requires C 77.74, H 8.70, N 8.63); ν_{max} (neat)/cm^{-1} 2970, 2932, 2915, 1640, 1596, 1448, 1262, 1196, 1131, 960, 907, 818, 727, 691, 642; δ_H (300 MHz, $CDCl_3$) *δ* 7.34-7.21 (5H, m), 6.38 (1H, s), 6.30 (2H, s), 4.64 (2H, s), 4.05 (2H, s), 3.40 (2H, q, *J* 7.2 Hz), 3.28 (2H, q, *J* 7.2 Hz), 2.21 (6H, s), 1.18 (3H, t, *J* 7.2 Hz), 1.15 (3H, t, *J* 7.2 Hz); δ_C (75 MHz, $CDCl_3$) *δ* 168.6 (C), 149.8 (C), 139.4 (C), 138.7 (C), 128.6 (CH), 127.2 (CH), 127.0 (CH), 119.6 (CH), 110.9 (CH), 55.8 (CH_2), 51.5 (CH_2), 41.2 (CH_2), 40.5 (CH_2), 21.9 (CH_3), 14.4 (CH_3), 13.2 (CH_3); *m/z* (+ESI) 671 ([2*M* + Na]$^+$, 50%), 347 ([*M* + Na]$^+$, 100), 325 ([*M* + H]$^+$, 80).

2-((3,5-Dimethylphenyl)(4-methoxybenzyl)amino)-*N*,*N*-diethylacetamide (23)

Subjecting **18** (510 mg, 2.41 mmol) to the general procedure K afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **23** (642 mg, 82%) as a white solid. R_f 0.13 (hexane-EtOAc, 80:20); m.p. 96–97 °C; (Found: C 74.08, H 9.01, N 7.73; $C_{22}H_{30}N_2O_2$ requires C 74.54, H 8.53, N 7.90); ν_{max} (neat)/cm^{-1} 2961, 2929, 2912, 1643, 1599, 1509, 1353, 1241, 1196, 1167, 1135, 1034, 961, 826, 806, 770, 693; δ_H (300 MHz, $CDCl_3$) *δ* 7.21 (2H, d, *J* 8.6 Hz), 6.85 (2H, d, *J* 8.6 Hz), 6.38 (1H, s), 6.32 (2H, s), 4.57 (2H, s), 4.02 (2H, s), 3.78 (3H, s), 3.39 (2H, q, *J* 7.1 Hz), 3.25 (2H, t, *J* 7.1 Hz), 2.21 (s, 6H), 1.17 (3H, t, *J* 7.1 Hz), 1.11 (3H, t, *J* 7.1 Hz); δ_C (75 MHz, $CDCl_3$) *δ* 168.7 (C), 158.7 (C), 149.8 (C), 138.7 (C), 131.2 (C), 128.5 (CH), 119.6 (CH), 114.0 (CH), 111.0 (CH), 55.4 (CH_3), 55.0 (CH_2), 51.2 (CH_2), 41.2 (CH_2), 40.5 (CH_2), 21.9 (CH_3), 14.4 (CH_3), 13.2 (CH_3); *m/z* (+ESI) 731 ([2*M* + Na]$^+$, 40%, 377 ([*M* + Na]$^+$, 100), 355 ([*M* + H]$^+$, 70).

2-((3,5-Dimethylphenyl)(4-(2-fluoroethoxy)benzyl)amino)-*N*,*N*-diethylacetamide (24).

Subjecting **19** (820 mg, 3.00 mmol) to the general procedure K afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **24** (1 019 mg, 88%) as a colorless oil that crystallized on prolonged standing. R_f 0.33 (hexane-EtOAc, 70:30); m.p. 87–90 °C; (Found: C 71.26, H 8.35, N 7.15; $C_{23}H_{31}FN_2O_2$ requires C 71.47, H 8.08, N 7.25); ν_{max} (neat)/cm^{-1} 2972, 2929, 1649, 1597, 1509, 1451, 1375, 1245, 1135, 1074, 921, 882, 814, 729, 689; δ_H (500 MHz, $CDCl_3$) δ 7.24 (2H, d, *J* 8.6 Hz), 6.88 (2H, d, *J* 8.6 Hz), 6.41 (1H, s), 6.35 (2H, s), 4.74 (2H, dt, $^2J_{HF}$ 47.4 Hz, $^3J_{HH}$ 4.2 Hz), 4.59 (2H, s), 4.20 (2H, dt, $^3J_{HF}$ 27.8 Hz, $^3J_{HH}$ 4.2 Hz), 4.04 (2H, s), 3.40 (2H, q, *J* 7.1 Hz), 3.27 (2H, q, *J* 7.1 Hz), 2.22 (6H, s), 1.17 (3H, t, *J* 7.1 Hz), 1.12 (3H, t, *J* 7.1 Hz; δ_C (125 MHz, $CDCl_3$) 168.5 (C), 157.7 (C), 149.7 (C), 138.8 (C), 131.8 (C), 128.8 (CH), 120.1 (C), 114.9 (2 overlapping CH signals), 111.4 (C), 82.1 (d, $^1J_{CF}$ 170.6 Hz), 67.3 (d, $^2J_{CF}$ 20.5 Hz, CH_2), 55.3 (CH_2), 51.5 (CH_2), 41.3 (CH_2), 40.5 (CH_2), 21.9 (CH_3), 14.4 (CH_3), 13.2 (CH_3); δ_F (282 MHz, $CDCl_3$) δ –223.87 (m, 1F); *m*/*z* (+ESI) 795 ([2*M* + Na]$^+$, 15%, 409 ([*M* + Na]$^+$, 100), 387 ([*M* + H]$^+$, 45).

2-((3,5-Dimethylphenyl)(4-isopropoxybenzyl)amino)-*N*,*N*-diethylacetamide (25)

Subjecting **20** (808 mg, 3.00 mmol) to the general procedure K afforded, following purification by flash chromatography (hexane-EtOAc, 70:30), gave **25** (989 mg, 86%) as a crystalline solid. R_f 0.34 (hexane-EtOAc, 70:30); m.p. 87–90 °C; (Found: C 74.61, H 9.55, N 7.16; $C_{24}H_{34}N_2O_2$ requires C 75.35, H 8.96, N 7.32); ν_{max} (neat)/cm^{-1} 2974, 2921, 1643, 1597, 1509, 1463, 1371, 1241, 1195, 1119, 951, 813, 772, 689; δ_H (500 MHz, $CDCl_3$) δ 7.21 (2H, d, *J* 8.5 Hz), 6.85 (2H, d, *J* 8.5 Hz), 6.40 (1H, s), 6.34 (2H, s), 4.58 (2H, s), 4.20 (1H, quin., *J* 6.1 Hz), 4.04 (2H, s), 3.41 (2H, q, *J* 7.1 Hz), 3.27 (2H, q, *J* 7.1 Hz), 2.24 (6H, s), 1.34 (6H, d, *J* 6.1 Hz), 1.18 (3H, t, *J* 7.1 Hz), 1.13 (3H, t, *J* 7.1 Hz); δ_C (125 MHz, $CDCl_3$) δ 168.7 (C), 157.0 (C), 149.8 (C), 138.6 (C), 131.0 (C), 128.5 (CH), 119.5 (CH), 116.0 (CH), 110.9 (CH), 70.0 (CH), 55.0 (CH_2), 51.1 (CH_2), 41.2 (CH_2), 40.5 (CH_2), 22.2 (CH_3), 21.9 (CH_3), 14.4 (CH_3), 13.2 (CH_3); *m*/*z* (+ESI) 787 ([2*M* + Na]$^+$, 30%), 405 ([*M* + Na]$^+$, 100), 383 ([*M* + H]$^+$, 70).

2-((3,5-Dimethylphenyl)(4-(benzyloxy)benzyl)amino)-*N*,*N*-diethylacetamide (26)

Subjecting **21** (952 mg, 3.00 mmol) to the general procedure K afforded, following purification by flash chromatography (hexane-EtOAc, 80:20), gave **26** (1 074 mg, 83%) as a crystalline solid. R_f 0.48 (hexane-EtOAc, 70:30); m.p. 110–112 °C; (Found: C 77.54, H 8.03, N 6.42, $C_{28}H_{34}N_2O_2$ requires C 78.10, H 7.96, N 6.51); ν_{max} (neat)/cm^{-1} 2968, 2931, 1643, 1597, 1509, 1461, 1382, 1248, 1198, 1168, 1130, 1036, 956, 818, 771, 737, 693; δ_H (500 MHz, $CDCl_3$) δ 7.45 (2H, d, *J* 7.3 Hz), 7.40 (2H, t, *J* 7.3 Hz), 7.34 (1H, t, *J* 7.3 Hz), 7.24 (2H, d, *J* 8.6 Hz), 6.95 (2H, d, *J* 8.6 Hz), 6.41 (1H, s), 6.34 (2H, s), 5.07 (2H, s), 4.60 (2H, s), 4.04 (2H, s), 3.42 (2H, q, *J* 7.1 Hz), 3.28 (2H, q, *J* 7.1 Hz), 2.24 (6H, s), 1.19 (3H, t, *J* 7.1 Hz), 1.14 (3H, t, *J* 7.1 Hz); δ_C (125 MHz, $CDCl_3$) δ 168.7 (C), 158.0 (C), 149.8 (C), 138.7 (C), 137.2 (C), 131.5 (C), 128.7 (CH), 128.5 (CH), 128.0 (CH), 127.6 (CH), 119.6 (CH), 115.0 (CH), 111.0 (CH), 70.2 (CH_2), 55.1 (CH_2), 51.3 (CH_2), 41.2 (CH_2), 40.5 (CH_2), 21.9 (CH_3), 14.4 (CH_3), 13.2 (CH_3); *m/z* (+ESI) 453 ([*M* + Na]$^+$, 100%), 431 ([*M* + H]$^+$, 45).

1.3. Biological Experimental

1.3.1 Membrane preparation

HEK-293 cells stably expressing A147T and WT TSPO were generated, and harvested for membrane preparation, as per Sokias et al.[34] In brief, A147T and WT-expressing HEK-293 cells were incubated with EDTA (0.04% w/v) in phosphate buffered saline for 10 min (37 ^oC, 5% CO2), and spun at 172 *g* for 5 min. The cells were resuspended in 3 ml of Tris-HCl (5 mM, pH 7.4) and homogenised (Ultra-Turrax homogeniser; IKA Werke, Staufen, Germany) on ice. The homogenate was centrifuged at 48,000 *g* at 4 ^oC for 15 min, and the pellet was resuspended in 10 ml of Tris-HCl (50 mM, pH 7.4). This was repeated twice, and a total protein count conducted using a BCA protein assay, as per the manufacturer's protocol (Pierce Biotechnology Inc., Rockford, IL, USA).

1.3.2 Radioligand binding

Competition radioligand binding was performed as per Sokias et al.[34] Briefly, [^{3}H]PK 11195 (Perkin-Elmer, Waltham, MA, USA) was used as the competing ligand due to its commercial

availability and ability to bind with similar affinity at WT and A147T TSPO. For 90 min in a final volume of 200 µL, membranes (20 µg/well) were incubated in duplicate with ~K_d concentration (10 nM) of [^{3}H]PK 11195 in Tris-HCl (50 mM, pH 7.4), and with logarithmically-spaced concentrations of test compounds. To determine total binding, [^{3}H]PK 11195 and membranes were incubated with vehicle control, 0.1% DMSO (v/v) in 50 mM Tris-HCl (pH 7.4). Non-specific binding was established by the incubation of [^{3}H]PK 11195 and membranes with unlabelled PK 11195 (1 µM; Sigma-Aldrich).

After 90 min, the reaction was terminated by rapid filtration through a glass-fibre filter plate (Millipore, Carrigtwohill, Ireland) and washed with ice-cold Tris-HCl (50 mM, pH 7.4) using a Brandel 96-sample vacuum harvester (Gaithersburg, MD, USA). The filter plate retaining the receptor-bound radioligand was allowed to dry overnight, followed by the addition of Microscint-0 scintillation cocktail (Perkin-Elmer). Emitted photons were counted by a Microbeta^2 2450 Microplate Counter (Perkin-Elmer). Total counts per minute were corrected for non-specific binding, and the resulting specific binding at each concentration was expressed as a percentage of the maximum amount of [^{3}H]PK11195 that can bind to TSPO, which was determined by the vehicle control. K_i was determined through a four-parameter non-linear regression curve fit of the data in GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, CA, U.S.A).