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Extracellular Loop 2 Of The Free Fatty Acid Receptor 2 Mediates Allosterism Of A Phenylacetamide Ago-Allosteric Modulator

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SUPPLEMENTARY METHODS

Compound Synthesis

General details

Commercially available reagents from Aldrich and Alfa Aesar chemical companies were generally used as supplied without further purification. Tetrahydrofuran (THF) was dried by distillation from sodium-benzophenone ketyl under argon. 'Light petroleum' refers to the fraction boiling between 40 °C and 60 °C. Dichloromethane was dried by distillation from calcium hydride. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Aldrich and used as supplied from Sure/Seal™ bottles. Reactions were routinely carried out under an inert atmosphere of argon or nitrogen. Analytical thin layer chromatography was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF_{254} (Art. 05554). Developed plates were visualized under ultra-violet light (254 nm) and/or alkaline potassium permanganate dip. Flash chromatography was performed using DAVISIL® silica (60 Å; 35-70 μM) from Fisher (cat. S/0693/60). Fully characterized compounds were chromatographically homogeneous.

Melting points were determined using a Stuart Scientific SMP10 apparatus and are uncorrected. Mass spectra were obtained on a Kratos Concept IS EI (electron impact) spectrometer. ^{1}H NMR spectra were recorded at 200 and 400 MHz on Bruker AC200 and DPX400 spectrometers; ^{13}C NMR spectra were recorded at 50 and 101 MHz on the same instruments. Chemical shifts are recorded in parts per million (δ in ppm) and are referenced against solvent signals (δ_C 77.16 for chloroform and δ_C 39.52 for methyl sulfoxide) for ^{13}C spectra and solvent residual resonances (δ_H 7.26 for chloroform and δ_H 2.50 methyl sulfoxide) for ^{1}H spectra.[1] Chemical shift values are accurate to ±0.01 ppm and ±0.1 ppm respectively. *J* values are given in Hz. Multiplicity designations used are: s, d, t, q, sept and m for singlet, doublet, triplet, quartet, septet and multiplet respectively. In ^{13}C NMR spectra, signals corresponding to CH, CH_2, or CH_3 groups are assigned from DEPT. Elemental analyses were carried out by the analytical service of the Chemistry Department at Heriot-Watt University using an Exeter CE-440 Elemental Analyser. Enantiomeric excess was

[1] H. E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.*, 1997, **62**, 7512-7515.

determined by chiral HPLC analysis using a Phenonenex Lux 3μ Cellulose-2 1000Å 150 x 4.6 mm column with 20% 2-propanol in hexane as the isocratic eluent and a flow of 0.2 ml/min with a column temperature of 30 ^{o}C.

(*rac*)-2-(4-chlorophenyl)-3-methyl-*N*-(thiazol-2-yl)butanamide (4-CMTB)

(*rac*)-2-(4-chlorophenyl)-3-methylbutanoic acid

A solution of *n*-BuLi (2.5 M / hexane; 220 mL, 550 mmol) was added dropwise to a stirred solution of (4-chlorophenyl)acetic acid (22.4 g, 131 mmol) in THF (350 mL) at –78 °C; the mixture was then warmed to 0 °C for 1 h. The reaction mixture was cooled to –78 °C and isopropyl iodide (42.0 mL, 420 mmol) was added. The mixture was allowed to come to ambient temperature over 18 h, resulting in partial precipitation of the lithium salt of 2-(4-chlorophenyl)-3-methylbutanoic acid. The latter was collected by filtration and the filtrate concentrated *in vacuo* to remove THF. The carboxylate salt was recombined with resulting residue and partitioned between dilute hydrochloric acid and EtOAc. The organic layer was washed successively with 10% sodium metabisufite solution, water and saturated brine; dried (Na_2SO_4) and evaporated. The crude product thus obtained was purified by flash column chromatography (100% CH_2Cl_2) to afford the *title compound* (17.9 g; 64%) as white powder: mp 93–96 °C (CH_2Cl_2); δ_H (200 MHz; $CDCl_3$) 10.88 (1 H, OH, broad s), 7.33 – 7.23 (4 H, m, chlorophenyl), 3.12 (1 H, d, *J* 10.6, α-CH), 2.29 (1 H, double septet, *J* 10.6 and 6.5, isopropyl CH), 1.07 (3 H, d, *J* 6.5, Me), 0.70 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 180.0 (C=O), 136.3 (aromatic C), 133.6 (aromatic C), 130.1 (aromatic CH×2), 128.9 (aromatic CH×2), 59.5 (α-CH), 31.9 (isopropyl CH), 21.6 (Me), 20.2 (Me); *m/z* (EI) 212 (M^{+} ^{35}Cl, 75%); (found: C, 62.29; H, 6.28. $C_{11}H_{13}ClO_2$ requires C, 62.12; H, 6.16%).

(*rac*)-2-(4-chlorophenyl)-3-methyl-*N*-(thiazol-2-yl)butanamide (4-CMTB)

A stirred mixture of (*rac*)-2-(4-chlorophenyl)-3-methylbutanoic acid (1.60 g; 7.52 mmol) and thionyl chloride (0.58 mL, 8.0 mmol) in anhydrous CH_2Cl_2 (10 mL) was heated at reflux under a nitrogen atmosphere. After 3 h the mixture was cooled and evaporated. The crude acid chloride was reconstituted with anhydrous CH_2Cl_2 (10 mL) and the resulting solution added dropwise under a nitrogen atmosphere into a stirred mixture of 2-aminothiazole (2.07 g, 20.7 mmol) and triethylamine (2.40 mL, 17.2 mmol) in anhydrous CH_2Cl_2 (30 mL). The resulting mixture was then heated under reflux. After 16 h the mixture was cooled to ambient temperature and washed successively with dilute hydrochloric acid, saturated sodium bicarbonate solution and saturated brine. The organic layer was dried (Na_2SO_4) and evaporated to afford a residue that was subjected to flash column chromatography (1:4 EtOAc/light petroleum), furnishing the *title compound* (1.56 g g; 70%) as white powder: mp 195–197 °C; δ_H (200 MHz; $CDCl_3$) 11.98 (1 H, broad s, NH), 7.56 (1 H, d, *J* 3.7, thiazole), 7.27 (4 H, app. s, chlorophenyl), 7.09 (1 H, d, *J* 3.7, thiazole), 3.21 (1 H, d, *J* 10.4, α-CH), 2.50 (1 H, double septet, *J* 10.4 and 6.5, isopropyl CH), 1.06 (3 H, d, *J* 6.5, Me), 0.79 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 171.7 (C=O), 160.0 (thiazole C-2), 136.5 (aromatic C), 136.4

(thiazole CH-4), 133.6 (aromatic C), 129.8 (aromatic CH×2), 129.0 (aromatic CH×2), 114.3 (thiazole CH-5), 60.8 (α-CH), 32.3 (isopropyl CH), 21.6 (Me), 20.6 (Me); *m/z* (EI) 294 (M^+ ^{35}Cl, 35%), 194 (57%), 167 (62%), 125 (100%); (found: C, 56.95; H, 5.12; N, 9.69. $C_{14}H_{15}ClN_2OS$ requires 57.04; H, 5.13; N, 9.50%).

(*R*)-(-)-2-(4-chlorophenyl)-3-methylbutanoic acid ((-)-CPA) and (*S*)-(+)-2-(4-chlorophenyl)-3-methylbutanoic acid ((+)-CPA)

Racemic (±)-2-(4-chlorophenyl)-3-methylbutanoic acid ((±)-CPA) was resolved using an adaption of a previously reported method.[2] Briefly, racemic 2-(4-chlorophenyl)-3-methylbutanoic acid ((±)-CPA) was resolved by crystallization with (S)-(−)-α-1-phenylethylamine ((-)-PEA) from 20% aqueous *n*-propanol. The obtained salt was recrystallized twice from aqueous n-propanol, the enantiomerically enriched *S*-(+)-CPA was obtained by dissolving the salt in dilute hydrochloric acid and extracting with dichloromethane. The organic phase was washed with brine, dried ($MgSO_4$) and concentrated. The residue was refined further by repeating the procedure twice to give *S*-(+)-CPA with >99% ee. The pure enantiomer *R*-(-)-CPA was obtained by a corresponding protocol. NMR spectra were in agreement with those reported for the racemic compound above.

(*S*)-(+)-2-(4-chlorophenyl)-3-methyl-*N*-(thiazol-2-yl)butanamide (*S*-4-CMTB)

A mixture of (*S*)-(+)-CPA (31.7 mg; 0.15 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC; 34.7 mg; 0.18 mmol), 1-hydroxybenzotriazole (HOBt; 25.6 mg; 0.19 mmol), 2-aminothiazole (17.9 mg; 0.18 mmol) and triethylamine (3 drops) in CH_2Cl_2 (20 mL) was stirred under an argon atmosphere for 4 days. The reaction mixture was diluted with CH_2Cl_2, washed with dilute hydrochloric acid, saturated sodium bicarbonate solution and saturated brine. The organic phase was dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography (SiO_2, 1:4 EtOAc/light petroleum) to give the *title compound* (19 mg; 43%, 98.1% ee) as white powder. NMR spectra were in agreement with those reported for the racemic compound above.

(*R*)-(-)-2-(4-chlorophenyl)-3-methyl-*N*-(thiazol-2-yl)butanamide (*R*-4-CMTB)

R-4-CMTB was prepared from (*R*)-(-)-CPA (26.3 mg; 0.12 mmol) and 2-aminothiazole (14.9 mg; 0.15 mmol) following the procedure described for S-4-CMTB to give the *title compound* (22.9 mg; 65%, 96.6% ee) as white powder. NMR spectra were in agreement with those reported for the racemic compound above.

2-(4-chlorophenyl)-*N*-(thiazol-2-yl)ethanamide (HWD001)

HWD001 was prepared as a white powder following the procedure described for preparation of 4-CMTB by reaction of (4-chlorophenyl)acetic acid with thionyl chloride followed by 2-aminothiazole: mp 202–207 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 12.56 (1 H, broad s, NH), 7.48 (1 H, d, *J* 3.6, thiazole), 7.36 – 7.24 (4 H, m, chlorophenyl), 7.03 (1 H, d, *J* 3.6,

[2] V. V. N. Reddy, K. Ishratullah, P. V. K. Raju, A. N. Rao, T. R. Rao, PCT Int. Appl. WO 2004/060850.

thiazole), 3.86 (2 H, s, α-CH_2); δ_C (50 MHz; $CDCl_3$) 168.6 (C=O), 159.8 (thiazole C-2), 136.6 (thiazole CH-4), 133.9 (aromatic C), 131.8 (aromatic C), 130.9 (aromatic CH×2), 129.3 (aromatic CH×2), 114.3 (thiazole CH-5), 42.3 (α-CH_2); *m/z* (EI) 252 (M^+ ^{35}Cl, 69%), 152 (82%), 125 (99%), 100 (100%); (found: C, 52.17; H, 3.47; N, 11.34. $C_{11}H_9ClN_2OS$ requires 52.28; H 3.59; N 11.08%).

(*rac*)-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)propanamide (HWD002)

HWD002 was prepared as a white powder following the procedure described for preparation of 4-CMTB by reaction of (*rac*)-2-(4-chlorophenyl)propanoic acid with thionyl chloride followed by 2-aminothiazole: mp 195-198 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 11.92 (1 H, broad s, NH), 7.46 (1 H, d, *J* 3.6, thiazole), 7.32 – 7.21 (4 H, m, chlorophenyl), 7.06 (1 H, d, *J* 3.6, thiazole), 3.85 (1 H, q, *J* 7.1, α-CH), 1.63 (3 H, d, *J* 7.1, Me); δ_C (50 MHz; $CDCl_3$) 172.0 (C=O), 160.0 (thiazole C-2), 138.4 (aromatic C), 136.6 (thiazole CH-4), 133.6 (aromatic C), 129.2 (aromatic CH×2), 128.9 (aromatic CH×2), 114.2 (thiazole CH-5), 46.2 (α-CH), 19.0 (Me); *m/z* (EI) 266 (M^+ ^{35}Cl, 81%), 166 (86%), 139 (100%); (found: C, 53.78; H, 4.18; N, 10.36. $C_{12}H_{11}ClN_2OS$ requires 54.03; H, 4.16; N, 10.50%).

(*rac*)-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)butanamide (HWD003)

HWD003 was prepared as a white powder following the procedure described for preparation of 4-CMTB by alkylation of (4-chlorophenyl)acetic acid with ethyl iodide and conversion of the resulting carboxylic acid into the acid chloride followed by reaction with 2-aminothiazole: mp 180–182 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 10.88 (1 H, broad s, NH), 7.45 (1 H, d, *J* 3.7, thiazole), 7.31 – 7.20 (4 H, m, chlorophenyl), 7.01 (1 H, d, *J* 3.5, thiazole), 3.50 (1 H, t, *J* 7.6, α-CH), 2.31 – 2.13 (1 H, m), 1.95 – 1.77 (1 H, m), 0.91 (3 H, t, *J* 7.3, Me); δ_C (50 MHz; $CDCl_3$) 171.5 (C=O), 159.7 (thiazole C-2), 137.0 (aromatic C), 136.6 (thiazole CH-4), 133.7 (aromatic C), 129.4 (aromatic CH×2), 129.2 (aromatic CH×2), 114.2 (thiazole CH-5), 54.2 (α-CH), 27.0 (CH_2), 12.4 (Me); *m/z* (EI) 280 (M^+ ^{35}Cl, 69%), 180 (85%), 153 (83%); (found: C, 55.44; H, 4.65; N, 9.78. $C_{13}H_{13}ClN_2OS$ requires 55.61; H, 4.67; N, 9.98%).

(*rac*)-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)pentanamide (HWD004)

HWD004 was prepared as a white powder following the procedure described for preparation of 4-CMTB by alkylation of (4-chlorophenyl)acetic acid with propyl iodide and conversion of the resulting carboxylic acid into the acid chloride followed by reaction with 2-aminothiazole: mp 127–131 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 12.26 (1 H, broad s, NH), 7.51 (1 H, d, *J* 3.7, thiazole), 7.29 – 7.19 (4 H, m, chlorophenyl), 7.07 (1 H, d, *J* 3.7, thiazole), 3.65 (1 H, t, *J* 7.6, α-CH), 2.28 – 2.08 (1 H, m, C*H*HC*H*$_2$$CH_3$), 1.93 – 1.74 (1 H, m, C*H*HC*H*$_2$$CH_3$), 1.42 – 1.14 (2 H, m, CH_2C*H*$_2$$CH_3$), 0.91 (3 H, t, *J* 7.2, CH_2CH_2C*H*$_3$); δ_C (50 MHz; $CDCl_3$) 171.7 (C=O), 160.2 (thiazole C-2), 137.3 (aromatic C), 136.4 (thiazole CH-4), 133.6 (aromatic C), 129.2 (aromatic CH×2), 129.1 (aromatic CH×2), 114.2 (thiazole CH-5), 52.2 (α-CH), 35.9 (CH_2), 21.0 (CH_2), 14.0 (Me); *m/z* (EI) 294 (M^+ ^{35}Cl, 73%), 194 (83%), 167 (68%), 100 (78%);

(found: C, 57.18; H, 5.21; N, 9.67. $C_{14}H_{15}ClN_2OS$ requires 57.04; H, 5.13; N, 9.50%).

(*rac*)-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)hexanamide (HWD005)

HWD005 was prepared as a white powder following the procedure described for preparation of 4-CMTB by alkylation of (4-chlorophenyl)acetic acid with butyl iodide and conversion of the resulting carboxylic acid into the acid chloride followed by reaction with 2-aminothiazole: mp 141–145 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 11.30 (1 H, broad s, NH), 7.49 (1 H, d, *J* 3.7, thiazole), 7.32 – 7.21 (4 H, m, chlorophenyl), 7.05 (1 H, d, *J* 3.6, thiazole), 3.60 (1 H, t, *J* 7.6, α-CH), 2.32 – 2.08 (1 H, m, α-CHC*H*H), 1.96 – 1.75 (1 H, m, α-CHC*H*H), 1.43 – 1.12 (2 H, m, CH_2C$H_2$$CH_3$), 0.86 (3 H, t, *J* 6.9, Me); δ_C (50 MHz; $CDCl_3$) 171.7 (C=O), 160.0 (thiazole C-2), 137.3 (aromatic C), 136.5 (thiazole CH-4), 133.6 (aromatic C), 129.3 (aromatic CH×2), 129.2 (aromatic CH×2), 114.2 (thiazole CH-5), 52.5 (α-CH), 33.5 (CH_2), 29.9 (CH_2), 22.6 (CH_2), 14.0 (Me); *m/z* (EI) 308 (M^+ ^{35}Cl, 89%), 208 (84%), 125 (100%); (found: C, 58.18; H, 5.53; N, 9.17. $C_{15}H_{17}ClN_2OS$ requires C, 58.34; H, 5.55; N, 9.07%).

(*rac*)-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)heptanamide (HWD006)

HWD006 was prepared as a white powder following the procedure described for preparation of 4-CMTB by alkylation of (4-chlorophenyl)acetic acid with pentyl iodide and conversion of the resulting carboxylic acid into the acid chloride followed by reaction with 2-aminothiazole: mp 107–111 °C (EtOH); δ_H (200 MHz; $CDCl_3$) 11.82 (1 H, broad s, NH), 7.49 (1 H, d, *J* 3.6, thiazole), 7.31 – 7.17 (4 H, m, chlorophenyl), 7.04 (1 H, d, *J* 3.6, thiazole), 3.60 (1 H, t, *J* 7.6, α-CH), 2.31 – 2.05 (1 H, m, α-CHC*H*H), 1.96 – 1.71 (1 H, m, α-CHC*H*H), 1.37 – 1.11 (2 H, m, CH_2C$H_2$$CH_3$), 0.82 (3 H, app. t, *J* 6.0, Me); δ_C (50 MHz; $CDCl_3$) 171.6 (C=O), 159.9 (thiazole C-2), 137.3 (aromatic C), 136.6 (thiazole CH-4), 133.7 (aromatic C), 129.4 (aromatic CH×2), 129.2 (aromatic CH×2), 114.3 (thiazole CH-5), 52.6 (α-CH), 33.8 (CH_2), 31.7 (CH_2), 27.5 (CH_2), 22.6 (CH_2), 14.3 (Me); *m/z* (EI) 322 (M^+ ^{35}Cl, 81%), 222 (82%); (found: C, 59.31; H, 5.87; N, 8.56. $C_{16}H_{19}ClN_2OS$ requires C, 59.52; H, 5.93; N, 8.86%).

2-(4-chlorophenyl)-2-methyl-*N*-(thiazol-2-yl)propanamide (HWD007)

HWD007 was prepared as a white powder following the general procedures described for preparation of 4-CMTB but commencing with alkylation of 2-(4-chlorophenyl)propanoic acid with methyl iodide to afford 2-(4-chlorophenyl)-2-methylpropanoic acid; the latter was converted into the acid chloride by reaction with thionyl chloride and then into the *title compound* by subsequent reaction with 2-aminothiazole: mp 193–196 °C (EtOAc/light petroleum); δ_H (200 MHz; $CDCl_3$) 8.45 (1 H, broad s, NH), 7.39 – 7.25 (5 H, m, thiazole & chlorophenyl), 6.96 (1 H, d, *J* 3.4, thiazole), 1.66 (6 H, s, Me×2); δ_C (50 MHz; $CDCl_3$) 174.6 (C=O), 158.3 (thiazole C-2), 141.8 (aromatic C), 137.5 (thiazole CH-4), 133.9 (aromatic C), 129.4 (aromatic CH×2), 127.9 (aromatic CH×2), 114.0 (thiazole CH-5), 47.2 (α-C), 26.8 (Me×2); *m/z* (EI) 280 (M^+ ^{35}Cl, 36%), 153 (100%); (found: C, 55.01; H, 4.66; 10.11. $C_{13}H_{13}ClN_2OS$ requires C, 55.61; H, 4.67; N, 9.98%).

2-butyl-2-(4-chlorophenyl)-*N*-(thiazol-2-yl)hexanamide (HWD008)

HWD008 was prepared as a white powder following the general procedures described for preparation of 4-CMTB but commencing with alkylation of 2-(4-chlorophenyl)hexanoic acid with butyl iodide to afford 2-butyl-2-(4-chlorophenyl)hexanoic acid; the latter was converted into the acid chloride by reaction with thionyl chloride and then into the *title compound* by subsequent reaction with 2-aminothiazole: mp 146–149 °C (EtOAc/light petroleum); δ_H (200 MHz; $CDCl_3$) 8.44 (1 H, broad s, NH), 7.35 – 7.18 (5 H, m, thiazole & chlorophenyl), 6.96 (1 H, d, *J* 3.6, thiazole), 2.10 – 1.95 (4 H, m), 1.40 – 1.17 (4 H, m), 1.17 – 0.93 (4 H, m), 0.86 (6 H, t, *J* 7.2, Me×2); δ_C (50 MHz; $CDCl_3$) 174.0 (C=O), 158.4 (thiazole C-2), 140.6 (aromatic C), 137.6 (thiazole CH-4), 133.6 (aromatic C), 129.2 (aromatic CH×2), 128.6 (aromatic CH×2), 113.8 (thiazole CH-5), 54.1 (α-C), 34.6 (CH_2×2), 26.0 (CH_2×2), 23.2 (CH_2×2), 14.1 (Me×2); *m/z* (EI) 364 (M^+ ^{35}Cl, 72%), 237 (97%), 127 (100%); (found: C, 62.49; H, 6.94; N, 7.61. $C_{19}H_{25}ClN_2OS$ requires C, 62.53; H, 6.90; N, 7.68%).

(*rac*)-2-(4-chlorophenyl)-3-methyl-*N*-(pyridin-3-yl)butanamide (HWD009)

Following the procedures described for preparation of 4-CMTB, (*rac*)-2-(4-chlorophenyl)-3-methylbutanoic acid was converted into its acid chloride derivative and reacted with pyridin-3-amine to afford HWD009 as a white powder: mp 170–173 °C ($CHCl_3$/light petroleum); δ_H (200 MHz; $CDCl_3$) 8.48 (1 H, d, *J* 2.5, pyridine ring H-2), 8.32 (1 H, dd, *J* 1.4 and 4.7, pyridine ring H-6), 8.25 (1 H, ddd, *J* 1.4, 2.5 and 8.4, pyridine ring H-4), 7.47 (1 H, broad s, NH), 7.34 – 7.29 (4 H, m, chlorophenyl CH), 7.23 (1 H, dd, *J* 4.8 and 8.4, pyridine ring H-5), 3.00 (1 H, d, *J* 10.1, α-CH), 2.45 (1 H, double septet, *J* 10.1 and 6.5, isopropyl CH), 1.10 (3 H, d, *J* 6.5, Me), 0.74 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 172.5 (C=O), 144.5 (pyridine CH), 141.0 (pyridine CH), 137.0 (C), 135.5 (C), 133.5 (C), 129.5 (chlorophenyl CH×2), 128.5 (chlorophenyl CH×2), 127.8 (pyridine CH), 124.0 (pyridine CH), 61.7 (α-CH), 32.0 (isopropyl CH), 21.5 (Me), 20.5 (Me); *m/z* (EI) 288 (M^+ ^{35}Cl, 24%), 246 (37%), 167 (51%), 125 (99%).

(*rac*)-2-(4-chlorophenyl)-3-methyl-*N*-(pyridin-2-yl)butanamide (HWD011)

Following the procedures described for preparation of 4-CMTB, (*rac*)-2-(4-chlorophenyl)-3-methylbutanoic acid was converted into its acid chloride derivative and reacted with pyridin-2-amine to afford HWD011 as a white powder: mp 130–133 °C (light petroleum); δ_H (200 MHz; $CDCl_3$) 8.98 (1 H, broad s, NH), 8.30 – 8.21 (2 H, m, pyridine ring H-3 and H-6), 7.80 (1 H, ddd, *J* 1.9, 7.5 and 8.5, pyridine ring H-4), 7.28 – 7.18 (4 H, m, chlorophenyl CH), 7.07 (1 H, ddd, *J* 1.1, 4.9 and 7.3, pyridine ring H-5), 2.93 (1 H, d, *J* 10.4, α-CH), 2.45 (1 H, double septet, *J* 10.3 and 6.5, isopropyl CH), 1.05 (3 H, d, *J* 6.5, Me), 0.68 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 171.5 (C=O), 151.5 (pyridine ring C-2), 147.0 (pyridine ring CH-6), 138.0 (pyridine ring CH-4), 136.5 (chlorophenyl C), 132.5 (chlorophenyl C), 128.5 (chlorophenyl CH×2), 128.0 (chlorophenyl CH×2), 119.5 (pyridine ring CH-5), 114.0 (pyridine ring CH-3), 61.2 (α-CH), 31.0 (isopropyl CH), 21.0 (Me), 20.0 (Me); *m/z* (EI) 288 (M^+ ^{35}Cl, 36%), 273 (100%), 167 (9%), 125 (44%); (found: C, 66.56; H, 6.07; N, 9.70. $C_{16}H_{17}ClN_2O$

requires C, 66.55; H, 5.93; N, 9.70%).

(*rac*)-2-(4-chlorophenyl)-3-methyl-*N*-(pyridin-4-yl)butanamide (HWD012)

Following the procedures described for preparation of 4-CMTB, (*rac*)-2-(4-chlorophenyl)-3-methylbutanoic acid was converted into its acid chloride derivative and reacted with pyridin-4-amine to afford HWD012 as a white powder: mp 164–167 °C ($CHCl_3$/light petroleum); δ_H (200 MHz; $CDCl_3$) 8.40 (2 H, ~dd, *J* 4.9 and 1.5, pyridine ring CH×2), 7.94 (1 H, broad s, NH), 7.40 (2 H, ~dd, *J* 4.9 and 1.5, pyridine ring CH×2), 7.31 – 7.28 (4 H, m, chlorophenyl CH), 3.01 (1 H, d, *J* 10.1, α-CH), 2.45 (1 H, double septet, *J* 10.1 and 6.5, isopropyl CH), 1.09 (3 H, d, *J* 6.5, Me), 0.72 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 172.5 (C=O), 151.0 (pyridine CH-2/6), 145.5 (pyridine ring C-4), 137.0 (chlorophenyl C), 133.7 (chlorophenyl C), 130.5 (chlorophenyl CH×2), 129.5 (chlorophenyl CH×2), 114.5 (pyridine CH-3/5), 62.5 (α-CH), 32.0 (isopropyl CH), 22.0 (Me), 20.5 (Me); *m/z* (EI) 288 (M^+ ^{35}Cl, 40%), 246 (54%), 167 (78%), 125 (83%).

(*rac*)-2-(4-chlorophenyl)-*N*,3-dimethyl-*N*-(thiazol-2-yl)butanamide (HWD013)

To an ice-cooled suspension of NaH (60% w/w dispersion in mineral oil; 0.34 g, 8.5 mmol) in DMF (20 mL) was added 2-(4-chlorophenyl)-3-methyl-*N*-(thiazol-2-yl)butanamide (0.54 g, 1.8 mmol). After 20 minutes, methyl iodide (0.82 mL, 13 mmol) was added dropwise to the resulting mixture. The mixture was then left stirring overnight at ambient temperature. DMF was removed using cold finger apparatus and the solid residue partitioned between EtOAc (55 mL) and saturated NaCl solution (50 mL). The organic layer collected was dried (Na_2SO_4) and evaporated to dryness. The crude product thus obtained was purified by flash column chromatography (CH_2Cl_2) and crystallised (hexane) to afford product the *title compound* (0.26 g; 46%) as a white powder: mp 101–104 °C; δ_H (200 MHz; $CDCl_3$) 7.48 (1 H, d, *J* 3.6, thiazole), 7.27 (4 H, app. s, chlorophenyl), 6.99 (1 H, d, *J* 3.6, thiazole), 3.73 (3 H, s, N-Me), 3.58 (1 H, d, *J* 10.1, α-CH), 2.56 (1 H, double septet, *J* 10.1 and 6.5, isopropyl CH), 1.04 (3 H, d, *J* 6.5, Me), 0.73 (3 H, d, *J* 6.6, Me); δ_C (50 MHz; $CDCl_3$) 172.4 (C=O), 160.3 (thiazole C-2), 137.0 (thiazole CH-4), 136.0 (chlorophenyl C), 133.6 (chlorophenyl C), 130.0 (chlorophenyl CH×2), 129.2 (chlorophenyl CH×2), 115.2 (thiazole CH-5), 57.6 (α-CH), 35.0 (N-Me), 32.7 (isopropyl CH), 22.2 (Me), 20.3 (Me).