Supporting information

Imidazol-1-ylethylindazole voltage gated sodium (Na_v) channel ligands are neuroprotective during optic neuritis in a mouse model of multiple sclerosis.

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CONTENTS

Experimental

General. All starting materials were either commercially available or synthesized by methods reported in the literature. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F254, Merck), visualizing with ultraviolet light. Column chromatography was performed on silica gel Si II, Isolute columns. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin 300 Hz. ^{1}H NMR chemical shifts are reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded on either a VG ZAB SE spectrometer (EI, FAB) or a Gilson-Finningan AQA LC-mass spectrometer using a C-18 column (Hypersil BDS 100 mm × 4.6 mm, 5 μm). Purification by reverse-phase HPLC (Gilson) used preparative C-18 columns (Hypersil PEP 100 mm × 21 mm, 5 μm). IR spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR spectrophotometer. The microwave experiments were run on a Biotage Initiator 60 microwave. All compounds were at least 95% pure as assayed by LCMS (electrospray +ve). A Micromass Quattro Micro mass spectrometer (S/N: QAA688, Waters Ltd, 7309740) was used for analysis of the metabolism studies.

Chemistry

The synthesis of ***tert*-Butyl-2-Amino-2-(hydroxyimino)ethylcarbamate** and **3-[3-*tert*-Butoxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl]indazole** have been reported and characterised in our previous work and were synthesised by that method.[1]

General synthesis of *N*1-Benzyl-Substituted Oxadiazoles: To a solution of **3-[3-*tert*-Butoxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl]indazole** (0.25 g, 0.79 mmol) in anhydrous DMF (5 mL) was added cesium carbonate (0.78 g, 2.4 mmol), which was stirred for 30 min. The heteroaromatic containing alkyl halide was then added (2.0 mmol), and the resulting solution was stirred overnight at room temperature under nitrogen. The solvent was removed in vacuo and the crude residue dissolved in ethyl acetate (20 mL). The organic phase was washed with water (20 mL) and brine (20 mL) and dried over magnesium sulfate. The solvent was removed in vacuo to give the crude product. Unless otherwise stated, the crude compounds were purified using column chromatography, eluting with 4:1 cyclohexane/ethyl acetate.

tert-Butyl(5-(1-(2-(1H-imidazol-1-yl)ethyl)-1H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methylcarbamate (16). Clear oil; 65% yield; δ_H (300 MHz, $CDCl_3$) 1.48 (9H, s, $(CH_3)_3$), 4.59-4.63 (4H, m, 2 x CH_2), 4.79 (2H, t, J = 5.8 Hz, NCH_2), 6.66 (1H, s, HC=CH), 6.92 (1H, s, HC=CH), 7.07-7.48 (4H, m, ArH + N=CH), 8.26 (1H, d, J = 8.2 Hz, ArH); δ_C (DMSO-d_6, 75.5 MHz) 170.0, 169.2, 155.6, 140.8, 137.3, 129.2, 128.4, 127.4, 123.6, 121.5, 120.3, 119.3, 110.5, 78.3, 49.8, 45.5, 36.0, 28.1; LC-MS-EI 410.4 ($M+H^+$).

tert-Butyl-N-[[5-[1-(2-pyridyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (18). δ_H (300 MHz, DMSO-d_6) 1.42 (9H, s, $3(CH_3)_3$), 4.52 (2H, s, CH_2), 7.49-7.66 (3H, m, ArH),

7.76 (1H, t, J = 8.2 Hz, ArH) 8.40 (1H, d, J = 7.3 ArH), 8.81 (1H, d, J = 6.0 Hz, ArH), 9.04 (2H, d, J = 5.3 Hz, ArH); δ_C (75.5 MHz, $CDCl_3$) 211.0, 170.5, 168.7, 166.7, 159.4, 158.9, 157.5. 140.5, 134.6, 129.4, 125.2, 124.2, 121.6, 118.8, 116.0, 37.0, 28.4 (3C), 22.6. LC-MS-EI 393.4 ($M+H^+$).

tert-Butyl-N-[[5-[1-[(5-methylisoxazol-4-yl)methyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (21). δ_H (300 MHz, $CDCl_3$) 1.47 (9H, s, $3(CH_3)_3$) 2.33 (3H, s, CH_3), 4.60 (2H, s, CH_2), 5.75 (2H, s, CH_2), 5.90 (2H, s, CH=N), 7.39 (1H, t, J = 6.3 Hz, ArH), 7.50 (1H, t, J = 7.2 Hz, ArH) 7.60 (1H, d, J = 8.1 Hz, ArH), 8.28 (1H, d, J = 7.9 Hz, ArH); δ_C (75.5 MHz, $CDCl_3$) 210.92, 170.87, 168.46, 158.93, 140.58, 133.22, 130.47, 128.07. 124.03, 122.97, 121.61, 114.35, 110.29, 100.92, 45.91, 28.35 (3C), 22.72, 12.28. LC-MS-EI 411.5 ($M+H^+$).

tert-Butyl-N-[[5-[1-[(4-pyrazol-1-ylphenyl)methyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (22). δ_H (300 MHz, $CDCl_3$) 1.48 (9H, s, $3(CH_3)_3$) 4.62 (2H, d, J = 5.7 Hz, CH_2) 5.78 (2H, s, CH_2), 6.44 (1H, s, ArH), 7.31-7.51 (5H, m, ArH), 7.64 (1H, s, ArH), 7.67 (1H, s, ArH), 7.69 (1H, s, ArH) 7.88 (1H, d, J = 3.0 Hz, ArH), 8.31 (1H, , J = 7.8 Hz, ArH); δ_C (75 MHz, $CDCl_3$) 171.24, 168.48, 141.16, 140.40, 139.98, 133.44, 130.03, 128.55 (2), 127.74, 126.72, 123.74, 123.01, 121.60, 121.33, 119.42 (2), 118.49, 110.17, 107.83, 53.57, 36.91, 28.34 (3). LC-MS-EI 472.2 ($M+H^+$).

tert-Butyl-N-[[5-[1-(1,3-benzodioxol-5-ylmethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (23). δ_H (300 MHz, $CDCl_3$) 1.48 (9H, s, (OtBu), 4.61 (2H, d, J = 4.2 Hz, CH2NH), 5.64 (2H, s, NCH2), 5.91 (2H, s, OCH_2O), 6.73-6.83 (3H, m, ArH), 7.35-7.60 (4H, m, ArH), 8.31 (1H, d, J = 8.3 Hz, ArH); δ_C (75.5 MHz, $CDCl_3$) 171.4, 168.4, 148.2, 147.7, 140.4, 129.9, 129.1, 127.6, 123.7, 123.2, 121.6, 121.1, 110.3, 108.4, 108.0, 101.3, 54.2, 37.0, 28.4. LC-MS-EI 450.4 ($M+H^+$).

General Method for the Mitsunobu Synthesis of the *N*2-Benzyl- Substituted Oxadiazoles: To a solution of **3-[3-*tert*-Butoxycarbonylaminomethyl-1,2,4-oxadiazol-5-yl]indazole** (0.25 g, 0.79 mmol), under nitrogen, in toluene (10 mL), benzyl alcohol (0.87 mmol), tributylphosphine (0.26 g, 0.31 mL, 1.26 mmol), and TMAD (0.22 g, 1.26 mmol) were added successively. The resulting clear-yellow solution was allowed to stir overnight at room temperature. The solution was then extracted with water, 1 N HCl, 1 N sodium hydroxide (aq), and brine. The combined aqueous phases were washed with dichloromethane and the combined organic phases dried over magnesium sulfate. The solvent was removed in vacuo. Unless otherwise stated, the crude compounds were purified using column chromatography (4:1 cyclohexane/ethyl acetate)

tert-Butyl((5-(2-(2-(1H-imidazol-1-yl)ethyl)-2H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methyl)carbamate (17). White solid; 47% yield; mp 100-101^oC; δ_H (300 MHz, MeOH-d_4); 1.47 (9H, s, $(CH_3)_3$)), 4.46 (2H, s, CH_2), 4.63 (2H, t, J = 5.8 Hz, CH_2), 5.30 (2H, t, J = 5.8

Hz, CH2), 6.67 (1H, s, ArH), 6.74 (1H, s, ArH), 7.05-7.39 (3H, m, ArH), 7.69 (1H, d, J = 8.6 Hz, ArH), 7.95 (1H, d, J = 8.3 Hz, ArH); δ_C (75 MHz, MeOH-d_4) 170.0, 169.0, 158.2, 149.5, 138.7, 129.1, 128.5, 126.9, 124.0, 121.3, 121.1, 121.0, 119.2, 80.8, 54.4, 47.8 , 37.2, 23.2; LC-MS-EI 410.4 ($M+H^+$).

tert-Butyl-N-[[5-[2-(2-pyridylmethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (19). White solid; 73% yield; δ_H (300 MHz, DMSO-d_6) 8.48 (1H, d, J = 4.1 Hz, ArH), 8.20 (1H, d, J = 8.1 Hz, ArH), 7.89 (1H, d, J = 8.5 Hz), 7.78 (1H, d, J = 6.0 Hz, ArH), 7.62 – 7.37 (5H, m, ArH), 7.30 (1H, dd, J = 7.9, 5.2 Hz, ArH), 5.96 (2H, s, CH_2), 3.33 (s, 2H, CH_2), 1.40 (s, 9H, CH_3), EI-MS: Requires 406.16983, Found 406.17039 ($M+H^+$).

tert-butyl-N-[[5-[2-[2-(2-thienyl)ethyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]carbamate (20). White solid; 31% yield; δ_H (300 MHz, DMSO-d_6) 1.39 (9H, s, O^{t}Bu), 3.51 (2H, t, J = 7.2 Hz, CH_2N), 4.39 (2H, d, J = 5.9 Hz, NCH_2), 5.20 (2H, d, J = 7.0 Hz, NCH_2), 6.88 (2H, m, HC=CH), 7.43 (2H, m, ArH+HC=CH), 7.60 (1H, m, ArH), 7.85 (1H, d, J = 8.6 Hz, ArH), 8.08 (1H, d, J = 8.0 Hz, ArH); δ_C (75.5 MHz, DMSO-d_6) 168.84, 167.31, 155.58, 147.21, 139.02, 126.93, 126.93, 126.04, 125.50, 124.64, 122.35, 119.99, 118.86, 118.18, 78.34, 53.97, 36.01, 29.82, 28.10; FAB-MS: Requires 448.14192, Found 448.14225 ($M+Na^+$).

General Method for the Deprotection of the Boc-Protected Oxadiazoles: The boc-protected oxadiazoles (0.03 g-0.25 g) were dissolved in trifluoroacetic acid (1.9 mL, 95%), and then triisopropylsilane (0.05 mL, 2.5%) and water (0.05 mL, 2.5%) were added. The solution was allowed to stir for 18 h at room temperature. Ice-cold diisopropyl ether or diethyl ether was added until a white precipitate started to form. The white precipitate was then filtered off and allowed to dry. The amines were generally used directly in the acylation step.

[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (26). Clear oil; >99% yield; δ_H (300 MHz, DMSO-d_6) 8.92 (1H, s, ArH), 8.79 (2H, s, NH), 8.26 (1H, d, J = 8.2 Hz, ArH), 7.83 (1H, d, J = 8.6 Hz, ArH), 7.68 (1H, d, J = 3.3 Hz, ArH), 7.64 – 7.52 (2H, m, ArH), 7.51 – 7.39 (1H, m, ArH), 5.74 (1H , s), 5.17 – 5.11 (2H, t, J = 5.6 Hz, CH_2), 4.80 (2H, t, J = 5.6 Hz, CH_2), 4.45 (2H, s, CH_2); δ_C (75 MHz, DMSO-d_6) 170.39, 165.70, 140.75, 135.93, 129.17, 127.83, 124.03, 122.16, 121.73, 120.60, 120.17, 113.58, 110.61, 48.85, 47.96, 34.18; LC-MS-EI: 310.4 ($M+H^+$).

[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (27). White powder; >99% yield; mp 105-106^{o}C; δ_H (300 MHz, DMSO-d_6) 8.91 (1H, s, ArH), 8.85 (2H, s, NH_2), 8.15 (d, J = 8.8 Hz, 1H, ArH), 7.80 (1H, d, J = 8.9 Hz, ArH), 7.62 (1H, s, ArH), 7.56 (1H, s, ArH), 7.47 (1H, dd, J = 6.8, 2.8 Hz, ArH), 5.51 – 5.36 (1H, m, CH_2), 4.94 – 4.84 (1H, m, CH_2), 4.49 (2H, s, CH_2); δ_C (75 MHz, DMSO-d_6) 167.65, 165.21, 158.61, 147.37, 136.04, 127.36, 126.14, 122.52, 120.26, 118.91, 118.29, 113.57, 52.78, 47.90, 34.09; CI-MS: Requires 310.14108, Found 310.14088 ($M+H^+$).

[5-[1-(2-Pyridyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (28). δ_H (300 MHz, DMSO-d_6) 9.04 (2H, d, J = 4.8 Hz, ArH), 8.85 (2H, s, NH_2), 8.79 (2H, d, J = 8.7 Hz, ArH), 8.40 (1H, d, J = 8.1 Hz, ArH), 7.76 (1H, t, J = 7.8 Hz, ArH), 7.66 – 7.56 (2H, m, ArH), 4.52 (2H, s, CH_2); δ_C (75.5 MHz, DMSO-d_6) 170.11, 165.94, 159.42, 158.10, 156.59, 139.86, 132.85, 129.55, 126.25, 125.31, 123.34, 120.92, 119.88, 115.85, 34.25. LC-MS-EI: 293.3 ($M+H^+$).

[5-[2-(2-pyridylmethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (29). White powder; >99% yield; δ_H (300 MHz, DMSO-d_6) 8.75 (2H, s, NH_2), 8.48 (1H, d, J = 4.8 Hz, ArH), 8.27 (1H, d, J = 8.1 Hz, ArH), 7.93 (1H, d, J = 8.5 Hz, ArH), 7.80 (1H, td, J = 7.7, 1.9 Hz, ArH), 7.59 (1H, t, J = 7.5 Hz, ArH), 7.48 (1H, q, J = 7.0 Hz, ArH), 7.39 – 7.23 (2H, m, ArH), 5.99 (2H, s, CH_2), 4.45 (3H, s, CH_2); δ_C (75 MHz, DMSO-d_6) 170.68, 165.64, 155.32, 149.31, 141.02, 137.36, 127.77, 127.23, 128.64, 123.93, 123.13, 122.08, 120.45, 110.98, 54.52, 34.21; EI-MS: Requires 306.11740, Found 306.11785 ($M+H^+$).

(5-(2-(2-(Thiophen-2-yl)ethyl)-2H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methanamine (30). White powder; 75% yield; δ_H (300 MHz, DMSO-d_6) 8.77 (2H, br s, NH2), 8.23 (1H, d, J = 7.5 Hz, ArH), 7.89 (1H, d, J = 7.5 Hz, ArH), 7.46 (2H, m, ArH), 7.29 (1H, m, HC=CH), 6.86 (2H, m, HC=CH), 5.24 (2H, t, J = 7.1 Hz, NCH_2), 4.47 (2H, s, NCH_2), 3.55 (2H, t, J = 7.0 Hz, CH_2N), δ_C (75 MHz, DMSO-d_6) 168.83, 165.32, 147.27, 139.03, 127.07, 126.78, 126.07, 125.84, 124.69, 122.60, 119.97, 118.51, 118.33, 54.07, 34.26, 29.78. EI-MS: Requires 326.1053, Found 326.1076 ($M+H^+$).

[5-[1-[(5-Methylisoxazol-4-yl)methyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (31). δ_H (300 MHz, DMSO-d_6) 2.34 (3H, s, CH_3), 4.46 (2H, s, CH_2), 5.96 (2H, s, CH_2), 6.14 (1H, s, CH=N), 7.49 (1H, t, J = 5.5 Hz, ArH), 7.64 (1H, t, J = 6.1 Hz, ArH) 7.97 (1H, d, J = 7.4 ArH), 8.28 (1H, d, J = 8.10 Hz, ArH), 8.71 (3H, s, NH_2); δ_C (75.5 MHz, DMSO-d_6) 205.76, 170.53, 165.71, 159.42, 140.60, 128.98, 127.99, 124.11, 121.98, 120.58, 111.25, 101.16, 45.00, 34.21, 11.67; LC-MS-EI 311.2 ($M+H^+$).

[5-[1-(4-Pyrazol-1-ylphenyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (32). δ_H (300 MHz, DMSO-d_6) 8.89 (2H, s, NH2), 8.44 (1H, s, ArH), 8.28 (1H, s), 8.03 (1H, s, ArH), 7.65 (7H, m, ArH), 6.51 (1H, s, ArH), 5.92 (2H, s, NCH_2), 4.46 (2H, s, NCH_2); δ_C (75 MHz, DMSO-d_6) 170.65, 165.68, 141.01, 140.38, 139.32, 133.93, 128.96 (2C), 128.62, 127.84, 127.70, 124.01, 122.11 (2C), 120.60, 118.60, 111.36, 107.87, 52.36, 34.21. LC-MS-EI 373.3 ($M+H^+$).

[5-[1-(1,3-Benzodioxol-5-ylmethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methanamine (33). δ_H (300 MHz, DMSO-d_6) 4.44 (2H, s, NCH_2), 5.66 (2H, s, NCH_2), 5.95 (2H, s, OCH_2O), 6.93-6.84 (3H, m, ArH), 7.47 (1H, d, J = 7.5, ArH), 7.56 (1H, d, J = 7.5, ArH), 8.00 (1H, d *J* 8.10 Hz, ArH), 8.24 (1H, d *J* 8.10 Hz, ArH), 8.84 (2H, br s, NH2); δ_C (75.5 MHz, DMSO-d_6) 170.71, 165.41, 147.40, 146.99, 140.15, 129.70, 128.42, 127.71, 123.93, 122.11, 121.44, 120.52, 111.38, 108.29, 108.22, 101.09, 52.72, 34.04. LC-MS-EI 350.3 ($M+H^+$).

Benzamide analogues

N-((5-(2-(2-(1H-Imidazol-1-yl)ethyl)-2H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methyl)benzamide (34). Off-white powder; 26% yield; mp 167-168^{o}C; δ_H (300 MHz, MeOH-d_4) 4.60 (1H, t *J* 6.0 Hz, NCH_2), 4.73 (1H, d *J* 5.8 Hz, CH_2NH), 5.30 (1H, t *J* 6.0 Hz, NCH_2), 6.67 (1H, s, NCH), 6.93 (1H, s, NCH), 7.33 (1H, s, NCHN), 7.39-7.56 (4H, m, ArH), 7.83 (1H, d *J* 8.3 Hz, ArH), 7.93 (2H, d *J* 8.4 Hz, ArH), 8.06 (1H, d *J* 7.9 Hz, ArH), 9.28 (1H, t *J* 5.7 Hz); δ_C (75 MHz, $CDCl_3$) 168.1, 167.6, 148.6, 137.6, 133.6, 131.9, 129.2, 128.6, 127.7, 127.4, 126.2, 123.0, 120.6, 120.1, 119.4, 118.3, 53.3, 47.1, 35.7; EI-MS: Requires 436.1498, Found 436.1520 ($M+Na^+$).

N-((5-(2-(pyridin-2-ylmethyl)-2H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methyl)benzamide (35) (83%N2 + 17%N1)
White powder (0.065g, 24%); δH (300 MHz, DMSO-d6) 4.67 (2H, d J 5.81 Hz, NCH2-isomer N1), 4.72 (2H, d J 5.70 Hz, NCH2-isomer N2), 5.96 (2H, s, CH2-isomer N2), 6.32 (2H, s, CH2-isomer N1), 7.26 (2H, m, ArH-isomer N2), 7.30 (4H, m, ArH-isomer N1), 7.49 (7H, m, ArH-isomer N2), 7.76 (1H, m, ArH-isomer N2), 7.84 (1H-isomer N1+1H-isomer N2, m, ArH-isomer N1+N2), 7.90 (2H-isomer N2+4H-isomer N1, t J 7.50 Hz, ArH-isomer N1+N2), 8.17 (2H-isomer N1+1H-isomer N2, m, ArH-isomer N1+N2), 8.35 (1H, d J 5.35Hz, ArH-isomer N1), 8.47 (1H, d J 4.82 Hz, ArH-isomer N2), 9.22 (1H, t J 5.89Hz, NH-isomer N1), 9.27 (1H, t J 5.89 Hz, NH-isomer N2); δC (75.5 MHz, DMSO-d6) 170.14, 168.87, 166.47, 155.52, 149.36, 149.36, 140.93, 137.20, 133.66, 131.49, 129.10, 128.35, 128.35, 127.59, 127.28, 127.28, 123.73, 123.02, 121.88, 120.45, 111.40, 54.51, 35.23; LC-MS-EI 411.3 (MH+,100); Found (CI) 411.15692 $C_{23}H_{18}N_6O_2$ (M+H) requires 411.15695.

N-[[5-[2-[2-(2-Thienyl)ethyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (36). White powder; 40% yield; δ_H (300 MHz, DMSO-d_6) 3.48 (2H, t *J* 7.34 Hz, CH_2CH_2N), 4.74 (2H, d *J* 5.73 Hz, NCH_2), 5.20 (2H, t *J* 7.17 Hz, NCH_2CH_2), 6.80 (2H, m, HC=CH), 7.20 (1H, m, HC=CH), 7.48 (5H, m, ArH), 7.91 (3H, m, ArH), 8.08 (1H, d *J* 7.98 Hz, ArH), 9.27 (1H, t *J* 5.68 Hz, NH); δ_C (75.5 MHz, DMSO-d_6) 168.55, 166.50, 147.24, 138.98, 138.98, 133.68, 131.51, 128.36, 128.36, 127.29, 126.89, 126.89, 125.98, 125.58, 124.59, 122.36, 119.96, 118.83, 118.21, 114.75, 53.96, 35.14, 29.85. CI-MS: Requires 430.13376, Found 430.13256 ($M+H^+$).

N-[[5-[1-[(5-Methylisoxazol-4-yl)methyl]indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (37). δ_H (300 MHz, DMSO-d_6) 2.32 (3H, s, CH_3), 4.73 (2H, s, CH_2), 5.93 (2H, s, CH_2), 6.10 (1H, s, CH=N), 7.52 (5H, m, ArH), 7.92 (3H, m, ArH) 8.18 (1H, d, J 8.6 ArH), 9.27 (1H, t, *J* 5.1 Hz, ArH), δ_C (75 MHz, DMSO-d_6) δ 224.76, 170.50, 169.98, 168.92, 166.48, 159.49, 140.50, 133.66, 131.50, 129.44, 128.35, 127.28, 123.92, 121.90, 120.57, 111.08, 101.12, 44.90, 35.24, 11.65. LC-MS-EI 415.3 ($M+H^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2,3,4-trimethoxy-benzamide (38). Yellow powder; δ_H (300 MHz, DMSO-d_6) 8.84 (t, *J* = 5.8 Hz,

1H, NH), 8.14 (d, *J* = 8.1 Hz, 1H, ArH), 7.64 (d, *J* = 8.5 Hz, 1H, ArH), 7.56 (d, *J* = 8.9 Hz, 1H, ArH), 7.51 – 7.44 (m, 1H, ArH), 7.42 – 7.27 (m, 2H, ArH), 7.01 (s, 1H, ArH), 6.93 (d, *J* = 8.9 Hz, 1H, ArH), 6.71 (s, 1H, ArH), 4.96 (t, *J* = 5.9 Hz, 2H, CH_2), 4.75 (d, *J* = 5.8 Hz, 2H, CH_2), 4.55 (t, *J* = 5.8 Hz, 2H, CH_2). δ_C (75.5 MHz, DMSO-d_6) 170.0, 168.9, 164.7, 155.9, 152.0. 141.5, 140.8, 137.3, 129.2, 128.4, 127.4. 125.3, 123.7, 121.5, 120.3, 119.6,, 199.3, 110.5, 107.9, 61.8, 60.5, 56.0, 49.8, 45.1, 35.3; EI-MS: Requires 526.1815, Found 526.1840 ($M+Na^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-1H-indole-4-carboxamide (39). δ_H (300 MHz, DMSO-d_6) δ 11.34 (s, 1H, NH), 8.98 (t, *J* = 5.7 Hz, 1H, NH), 8.16 (d, *J* = 8.1 Hz, 1H, ArH), 7.68 – 7.28 (m, 7H ArH), 7.17 (t, *J* = 7.7 Hz, 1H, ArH), 7.03 (s, 1H, ArH), 6.97 (s, 1H, ArH), 6.74 (s, 1H, ArH), 4.96 (t, *J* = 5.7 Hz, 2H, CH_2), 4.78 (d, *J* = 5.7 Hz, 2H, CH_2), 4.56 (t, *J* = 5.7 Hz, 2H, CH_2). δ_C (75 MHz, DMSO-d_6) δ 168.44, 162.88, 152.56, 150.68, 140.18, 136.80, 134.50, 129.98, 129.80, 128.61, 127.61, 127.26, 126.78, 124.21, 121.66, 120.71, 120.45, 120.04, 119.90, 113.06, 101.62, 48.89, 45.11, 40.29. LC-MS-EI 453.5 ($M+H^+$).

4-tert-Butyl-N-[[5-[1-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (40). White solid; 25% yield; δ_H (300 MHz, MeOH-d_4) 1.32 (9H, s, CH_3), 4.62 (2H, m, NCH_2), 4.81 (2H, m, NCH_2CH_2N), 4.87 (2H, m, NCH_2CH_2N), 6.76 (1H, s, HC=CH), 6.91 (1H, s, HC=CH), 7.34 (4H, m, HC=N + ArH), 7.51 (2H, d *J* 8.70 Hz, ArH), 7.86 (2H, m, ArH), 8.12 (1H, d *J* 8.09 Hz, ArH); δ_C (75.5 MHz, MeOH-d_4) 172.26, 170.39, 170.07, 156.71, 142.66, 138.44, 132.19, 131.54, 128.92, 128.85, 128.85, 128.42, 126.58, 126.58, 124.82, 123.45, 122.13, 120.86, 110.71, 51.14, 47.56, 36.71, 35.70, 35.82, 31.57; ESI-MS: Requires 492.21239, Found 492.21281 ($M+H^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2,3-dimethoxy-benzamide (41). White solid; 35% yield; δ_H (300 MHz, MeOH-d_4) 3.84 (6H, s, OCH_3), 4.67 (2H, m, NCH_2), 4.84 (2H, m, NCH_2CH_2N), 5.47 (2H, m, NCH_2CH_2N), 6.86 (1H, s, HC=CH), 7.00 (1H, s, HC=CH), 7.15 (6H, m, HC=N + ArH), 7.53 (1H, s, ArH), 8.05 (1H, d *J* 8.10 Hz, ArH); δ_C (75.5 MHz, DMSO-d_6) 170.04, 168.82, 167.80, 165.54, 152.95, 152.55, 147.41, 146.74, 140.81, 129.24, 127.40, 124.06, 123.78, 121.53, 121.03, 120.31, 119.33, 115.25, 110.49, 60.72, 55.79, 49.82, 45.49, 35.25; EI-MS: Requires 496.17190, Found 496.1722 ($M+H^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-(trifluoromethoxy)benzamide (42). White powder; 9% yield; δ_H (300 MHz, MeOH-d_4) 4.66 (2H, m, NCH_2), 4.83 (2H, m, NCH_2CH_2N), 5.02 (2H, m, NCH_2CH_2N), 6.75 (1H, s, HC=CH), 6.90 (1H, s, HC=CH), 7.41 (5H, m, HC=N + ArH), 7.60 (1H, t *J* 8.06 Hz, ArH), 7.83 (1H, s, ArH), 7.90 (1H, s, ArH), 7.92 (1H, d *J* 8.10 Hz, ArH), 8.20 (1H, d *J* 8.11 Hz, ArH); δ_C (75.5 MHz, DMSO-d_6) 170.14, 168.64, 164.90, 148.31, 140.82, 135.80, 130.68, 129.18, 127.41,

126.42, 126.42, 124.14, 124.14, 123.67, 123.67, 121.51, 120.29, 119.78, 118.29, 110.54, 49.83, 45.47, 35.29; ESI-MS: Requires 498.15015, Found 498.15124 ($M+H^+$).

4-Chloro-N-[[5-[2-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (43). δ_H (300 MHz, $CDCl_3$) 8.13 (d, *J* = 8.4 Hz, 1H, ArH), 8.03 (d, *J* = 8.7 Hz, 2H, ArH), 7.83 (d, *J* = 8.6 Hz, 1H, ArH), 7.75 (s, 1H, NH), 7.52 – 7.35 (m, 4H, ArH), 6.69 (s, 1H, ArH), 6.26 (s, 1H, ArH), 5.38 (t, *J* = 5.8 Hz, 2H, CH_2), 4.84 (d, *J* = 5.3 Hz, CH_2), 4.62 – 4.51 (t, *J* = 5.9 Hz, 2H, CH_2). δ_C (75.5 MHz, DMSO-d_6) δ 168.23, 167.21, 165.53, 147.33, 137.32, 136.39, 132.40, 129.23, 128.49, 128.27, 127.08, 125.70, 122.26, 119.96, 119.33, 119.22, 118.22, 53.27, 45.59, 35.14. LC-MS-EI 448.3 ($M+H^+$).

2,3-Difluoro-N-[[5-[2-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (44). Yellow Powder; δ_H (300 MHz, $CDCl_3$) 8.16 (d, *J* = 8.2 Hz, 1H, ArH), 7.96 – 7.77 (m, 2H, ArH), 7.52 – 7.17 (m, 8H, ArH), 6.84 (s, 1H, ArH), 6.64 (s, 1H, ArH), 5.40 (t, *J* = 6.1 Hz, 2H, CH_2), 4.92 (d, *J* = 5.5 Hz, 2H, CH_2), 4.61 (t, *J* = 6.1 Hz, 2H, CH_2). δ_C (75 MHz, DMSO-d_6) δ 167.94, 167.27, 163.08, 147.32, 137.33, 128.27, 127.07, 125.85, 125.70, 125.52, 125.03, 122.30, 119.97, 119.72, 119.50, 119.34, 119.19, 118.23, 53.32, 45.59, 35.11. EI-MS: Requires 450.1490, Found 450.1474 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-4-(trifluoromethoxy)benzamide (45). Orange powder; 20% yield; δ_H (500 MHz, DMSO-d_6) 4.60 (2H, t *J* 6.09 Hz, NCH_2), 4.75 (2H, d *J* 5.77 Hz, NCH_2CH_2N), 5.30 (2H, t *J* 6.06 Hz, NCH_2CH_2N), 6.66 (1H, s, HC=CH), 6.91 (1H, s, HC=CH), 7.31 (1H, s, N=CH), 7.46 (4H, m, ArH), 7.83 (1H, d *J* 8.57 Hz, ArH), 8.06 (3H, m, ArH), 9.40 (1H, t *J* 5.73 Hz, NH); δ_C (75 MHz, DMSO-d_6) δ 224.98, 168.22, 167.24, 165.36, 147.35, 137.32, 132.74, 129.67 (2C), 128.27, 125.72, 122.27 (2C), 120.69, 119.98, 119.23, 118.24, 115.24, 99.26, 89.79, 53.28, 45.60, 35.17. CI-MS: Requires 498.15015, Found 498.15158 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2-methyl-benzamide (46). Cream powder, δ_H (300 MHz, $CDCl_3$) 8.16 (d, *J* = 8.4 Hz, 1H, ArH), 7.83 (d, *J* = 8.6 Hz, 1H, ArH), 7.57 – 7.29 (m, 5H, ArH), 7.25(m, 2H, , ArH) 6.93 (s, 1H, NH), 6.80 (s, 1H, ArH), 6.52 (s, 1H, ArH), 5.42 (t, *J* = 6.0 Hz, 2H, CH_2), 4.87 (d, *J* = 5.7 Hz, 2H, CH_2), 4.60 (t, *J* = 6.0 Hz, 2H, CH_2), 1.25 (s, 3H, CH_3). LC-MS-EI 428.4 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-4-methoxy-benzamide (47). White solid; 18% yield; δ_H (300 MHz, DMSO-d_6) 3.81 (3H, s, OCH_3), 4.60 (2H, t *J* 5.89 Hz, NCH_2), 4.71 (2H, d *J* 5.89 Hz, NCH_2CH_2N), 5.30 (2H, t *J* 5.89 Hz, NCH_2CH_2N), 6.66 (1H, s, HC=CH), 6.93 (1H, s, HC=CH), 7.03(2H, d *J* 9.10 Hz, ArH), 7.31 (1H, s, N=CH), 7.43 (2H, m, ArH), 7.83 (1H, d *J* 8.03 Hz, ArH), 7.91 (2H, d, *J* 8.57 Hz, ArH), 8.06 (1H, d, *J* 8.57 Hz, ArH), 9.13 (1H, t *J* 5.89 Hz, NH); δ_C (75.5 MHz, DMSO-d_6) 168.51, 167.16, 166.62, 166.01, 147.34, 129.17, 129.17, 129.17, 128.30, 127.08, 125.87, 125.69, 122.25, 119.98, 119.34, 118.22, 113.57, 99.26, 55.31, 53.27, 45.60, 35.06; CI-MS: Requires 444.17841, Found 444.17997 ($M+H^+$).

4-Acetamido-N-[[5-[2-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzamide (48). δ_H (300 MHz, DMSO-d_6) δ 10.18 (s, 1H, NH), 9.14 (t, J = 5.8 Hz, 1H, NH), 8.07 (d, J = 7.9 Hz, 1H, ArH), 7.88 (d, J = 8.7 Hz, 2H, ArH), 7.82 (d, J = 8.3 Hz, 1H, ArH), 7.67 (d, J = 8.7 Hz, 2H, ArH), 7.53 – 7.30 (m, 3H, ArH), 7.00 (s, 1H, ArH), 6.75 (s, 1H, ArH), 5.31 (t, J = 6.0 Hz, CH_2), 4.71 (d, J = 5.7 Hz, 2H, CH_2), 4.63 (t, J = 5.9 Hz, 2H, CH_2), 2.06 (s, 3H, CH_3). LC-MS-EI 471.4 ($M+H^+$)

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]naphthalene-2-carboxamide (49). White powder, δ_H (300 MHz, DMSO-d_6) δ 9.46 (t, J = 5.7 Hz, 1H, NH), 8.55 (s, 1H, ArH), 8.14 – 7.91 (m, 2H, ArH), 7.84 (d, J = 8.2 Hz, 1H, ArH), 7.68 – 7.54 (m, 1H, ArH), 7.50 – 7.37 (m, 1H, ArH), 7.34 (s, 1H, ArH), 6.93 (s, 1H, ArH), 6.65 (s, 1H, ArH), 5.31 (t, J = 6.1 Hz, 1H, CH_2), 4.80 (d, J = 5.7 Hz, 1H, CH_2), 4.61 (t, J = 6.1 Hz, 1H, CH_2). 167.80, 148.53, 134.89, 132.07, 130.76, 129.07, 128.43, 128.20, 127.81, 127.69, 126.73, 126.14, 124.44, 123.86, 120.53, 120.01, 118.32, 47.00, 35.79, 29.72. EI-MS: Requires 464.1835, Found 464.1855 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2,3-dimethoxy-benzamide (50). δ_H (300 MHz, DMSO-d_6) δ 8.99 (t, J = 5.7 Hz, 1H, NH), 8.09 (d, J = 7.7 Hz, 1H, ArH), 7.83 (d, J = 8.1 Hz, 1H, ArH), 7.51 – 7.36 (m, 2H, ArH), 7.32 (s, 1H, ArH), 7.29 – 7.10 (m, 3H, ArH), 6.95 (s, 1H, ArH), 6.69 (s, 1H, ArH), 5.32 (t, J = 6.0 Hz, 2H, CH_2), 4.75 (d, J = 5.9 Hz, 2H, CH_2), 4.62 (t, J = 6.0 Hz, 2H, CH_2), 3.84 (s, 3H, CH_3) 3.81 (s, 3H, CH_3). LC-MS-EI 474.4 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-(trifluoromethoxy)benzamide (51). δ_H (300 MHz, $CDCl_3$) δ 8.18 (s, 1H, NH), 8.09 (d, J = 8.4 Hz, 1H, ArH), 7.99 (d, J = 7.7 Hz, 1H, ArH), 7.95 (s, 1H, ArH), 7.81 (d, J = 8.6 Hz, 1H, ArH), 7.54 – 7.30 (m, 5H, ArH), 6.69 (s, 1H, ArH), 6.30 (s, 1H, ArH), 5.37 (t, J = 5.5 Hz, 2H, CH_2), 4.82 (d, J = 5.1 Hz, 2H, CH_2), 4.55 (t, J = 5.5 Hz, 2H, CH_2). δ_C (75 MHz, DMSO-d_6) δ 237.95, 168.13, 167.26, 164.96, 151.20, 148.34, 147.35, 137.33, 135.81, 130.68, 128.25, 127.08, 126.41, 125.70, 124.15, 122.28, 119.96, 119.79, 119.23, 118.23, 53.29, 45.60, 35.20. LC-MS-EI 498.4 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-methyl-4-nitro-benzamide (52). Feathery yellow powder, δ_H (300 MHz, DMSO-d_6) δ 9.54 (t, J = 5.8 Hz, 1H, NH), 8.08 (t, J = 7.5 Hz, 2H, ArH), 8.02 (s, 1H, ArH), 7.94 (d, J = 8.4 Hz, 1H, ArH), 7.84 (d, J = 8.1 Hz, 1H, ArH), 7.52 – 7.35 (m, 2H, ArH), 7.32 (s, 1H, ArH), 6.92 (s, 1H, ArH), 6.67 (s, 1H, ArH), 5.31 (t, J = 6.0 Hz, 2H, CH_2), 4.76 (d, J = 5.7 Hz, 2H, CH_2), 4.61 (t, J = 6.0 Hz, 2H, CH_2), 2.54 (d, J = 6.6 Hz, 2H, CH_3). δ_C (75 MHz, DMSO-d_6) 168.1, 167.3, 165.0, 150.5, 147.4, 142.5, 137.4, 132.8, 131.7, 128.3, 127.1, 126.2, 125.7, 124.5, 122.3, 120.0, 199.3, 119.2, 118.3, 113.6, 53.3, 45.6, 19.3; EI-MS: Requires 473.1686, Found 473.1666 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-1,3-benzodioxole-5-carboxamide (53). White powder, δ_H (300 MHz, DMSO-d_6) δ 9.12 (t, J = 5.8 Hz, 1H, NH), 8.07 (d, J = 8.0 Hz, 1H, ArH), 7.84 (d, J = 8.3 Hz, 1H, ArH), 7.58 – 7.35 (m, 4H, ArH), 7.31 (s, 1H, ArH), 7.02 (d, J = 8.1 Hz, 1H, ArH), 6.92 (s, 1H, ArH), 6.67 (s, 1H, ArH), 6.10 (s, 2H, OCH_2), 5.30 (t, J = 6.1 Hz, 2H, CH_2), 4.70 (d, J = 5.7 Hz, 2H, CH_2), 4.60 (t, J = 6.1 Hz, 2H, CH_2). δ_C (75 MHz, $CDCl_3$) δ 211.08, 167.67, 166.86, 150.67, 148.58, 148.02, 137.69, 129.14, 127.76, 126.17, 122.94, 122.29, 120.56, 120.16, 119.37, 118.33, 108.06, 101.73, 53.36, 47.04, 35.74. EI-MS: Requires 458.1577, Found 458.1589 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-methyl-benzamide (54). White solid; 20% yield; δ_H (300 MHz, $CDCl_3$) 2.33 (3H, s, CH_3), 4.50 (2H, t J 5.88 Hz , NCH_2CH_2N), 4.82 (2H, d J 7.22 Hz, NCH_2), 5.31 (2H, t J 5.88 Hz, NCH_2CH_2N), 6.41 (1H, s, HC=CH), 6.72 (1H, s, HC=CH), 7.37 (4H, m, ArH + HC=N), 7.84 (3H, m, ArH), 8.03 (2H, m, ArH); δ_C (75.5 MHz, $CDCl_3$) 167.82, 167.82, 167.79,148.52, 138.49, 133.56, 132.62, 128.92, 128.49, 128.11, 127.67, 126.15, 124.34, 122.94, 120.53, 120.06, 119.37, 118.32, 53.24, 46.96, 35.70, 29.70, 21.33; ESI-MS: Requires 450.16635, Found 450.16544 ($M+Na^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2-methoxy-benzamide (55). White solid; 20% yield; δ_H (300 MHz, DMSO-d_6) 3.93 (3H, s, OCH_3), 4.73 (4H, d, J = 5.4 Hz, NCH_2), 4.78 (2H, d, J = 5.9 Hz, NCH_2), 5.36 (2H, t, J = 5.9 Hz, NCH_2), 7.06 (2H, m, HC=CH), 7.19 (1H, d, J = 8.0 Hz, ArH), 7.26 (1H, s, N=CH), 7.46 (3H, m, ArH), 7.82 (2H, m, ArH), 8.04 (1H, s, ArH), 8.10 (1H, m, ArH), 8.93 (1H, t, J = 5.9 Hz, NH); δ_C (75.5 MHz, DMSO-d_6) 206.92, 168.44, 167.18, 166.64, 165.24, 157.23, 147.33, 136.73, 132.78, 130.67, 127.17, 122.33, 121.83, 120.53, 120.02, 119.30, 118.21, 115.97, 112.13, 55.92, 53.08, 46.52, 35.37; ESI-MS: Requires 444.17841, Found 444.17988 ($M+H^+$).

N-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-methoxy-benzamide (56). White solid; 18% yield; δ_H (300 MHz, DMSO-d_6) 3.80 (3H, s, OCH_3), 4.60 (2H, d, J = 5.9 Hz, NCH_2), 4.73 (2H, d, J = 5.9 Hz, CH_2N), 5.30 (2H, t, J = 5.9 Hz, NCH_2), 6.66 (1H, s, HC=CH), 6.93 (1H, s, HC=CH), 7.12 (1H, m, ArH), 7.31 (1H, s, N=CH), 7.45 (5H, m, ArH), 7.84 (1H, d, J = 8.6 Hz, ArH), 8.07 (1H, d, J = 8.0 Hz, ArH), 9.28 (1H, t, J = 5.9 Hz, NH); δ_C (75.5 MHz, DMSO-d_6) 171.42, 168.34, 167.20, 166.27, 159.17, 147.34, 135.07, 129.53, 128.30, 125.71, 122.27, 119.99, 119.52, 119.25, 118.24, 117.44, 116.45, 115.16, 112.39, 55.23, 53.30, 45.61, 35.11; ESI-MS: Requires 444.17841, Found 444.17863 ($M+H^+$).

Sulfonamide analogues

N-((5-(1-(2-(1H-Imidazol-1-yl)ethyl)-1H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methyl)-2-methylbenzenesulfonamide (60). White powder; 39% yield; δ_H (300 MHz, MeOH-d_4) 2.58

(3H, s, CH_3), 4.32 (2H, s, NCH_2), 4.76 (2H, t, J = 5.2 Hz, NCH_2), 4.97 (2H, t, J = 4.9 Hz, NCH_2), 7.07 (1H, s, HC=CH), 7.09 (1H, s, HC=CH), 7.39 (3H, m, HC=N + ArH), 7.48 (2H, m, ArH), 7.89 (3H, m, ArH), 8.12 (2H, d, J = 4.3 Hz, ArH), 8.65 (1H, br s, NH); δ_C (75.5 MHz, DMSO-d_6) 169.78, 167.36, 146.20, 138.35, 135.45, 132.33, 132.10, 130.57, 129.16, 127.52, 126.42, 125.87, 124.97, 124.66, 123.61, 121.50, 120.48, 110.47, 49.32. LC-MS-EI 464.5 ($M+H^+$).

4-Chloro-N-[[5-[1-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzenesulfonamide (57). δ_H (300 MHz, DMSO-d_6) 8.72 (1H, t, J = 5.9 Hz, NH), 7.99 (1H, d, J = 8.1 Hz, ArH), 7.76 (2H, d, J = 8.5 Hz, ArH), 7.69 – 7.54 (2H, m, ArH), 7.54 – 7.25 (6H, m, ArH), 7.04 (1H, s, ArH), 6.76 (1H, s, ArH), 4.95 (2H, t, J = 5.7 Hz, CH_2), 4.57 (2H, t, J = 5.7 Hz, CH_2), 4.35 (2H, d, J = 5.8 Hz, CH_2); δ_C (75.5 MHz, DMSO-d_6) 169.89, 167.46, 140.78, 139.81, 137.29, 137.01, 128.91, 128.39, 127.41, 123.59, 121.46, 120.39, 119.30, 110.48, 49.82, 45.44, 37.94. LC-MS-EI 484.4 ($M+H^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2,3-dimethoxy-benzenesulfonamide (58). White powder; mp 197-198^oC; δ_H (300 MHz, DMSO-d_6), 8.39 (1H, t, J = 6.2 Hz, NH), 8.02 (1H, d, J = 8.1 Hz, ArH), 7.61 (1H, d, J = 8.5 Hz, ArH), 7.47 (1H, t, J = 7.7 Hz, ArH), 7.42 – 7.28 (3H, m, ArH), 7.24 (1H, d, J = 2.1 Hz, ArH), 7.03 (1H, s, ArH), 6.94 (1H, d, J = 8.5 Hz, ArH), 6.72 (1H, s, CH_2), 4.95 (2H, t, J = 5.8 Hz, CH_2), 4.55 (2H, t, J = 5.6 Hz, CH_2), 4.28 (2H, d, J = 6.1 Hz, CH_2), 3.73 (3H, s, OCH_3), 3.55 (3H, s, OCH_3); δ_C (75.5 MHz, DMSO-d_6) 169.8, 167.3, 151.8, 148.3, 140.8, 137.3, 131.8, 129.0, 128.4, 127.4, 123.5, 121.4, 120.3, 119.3, 113.5, 110.8, 110.4, 109.3, 55.5, 49.9, 45.5, 37.8, 24.9; ESI-MS: Requires 532.1379, Found 532.1398 ($M+Na^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-4 (trifluoromethoxy)benzenesulfonamide (59). White powder; mp 100-101^oC; δ_H (300 MHz, Acetone-d_6), 4.51 (2H, s, NCH_2), 4.77 (2H, t, J = 5.1 Hz, NCH_2), 5.03 (2H, t, J = 4.2 Hz, NCH_2), 6.86 (1H, s, HC=CH), 7.06 (1H, s, HC=CH), 7.25-7.61 (5H, m, ArH), 7.79 (1H, br s, NH), 8.03 (2H, d, J = 8.0 Hz ArH), 8.12 (1H, d, J = 7.5 Hz, ArH), 8.28 (1H, d, J = 7.7 Hz, ArH); δ_C (75.5 MHz, $CDCl_3$) 170.0, 167.3, 150.7, 140.7, 139.4, 137.2, 131.0, 129.1, 129.0, 127.4, 127.1, 123.6, 121.5, 121.3; ESI-MS: Requires 556.0991, Found 556.1008 ($M+Na^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-2-methyl-benzenesulfonamide (60). White powder; 39% yield; δ_H (300 MHz, MeOH-d_4) 2.58 (3H, s, CH_3), 4.32 (2H, s, NCH_2), 4.76 (2H, t, J = 5.2 Hz, NCH_2), 4.97 (2H, t, J = 4.9 Hz, NCH_2), 7.07 (1H, s, HC=CH), 7.09 (1H, s, HC=CH), 7.39 (3H, m, HC=N + ArH), 7.48 (2H, m, ArH), 7.89 (3H, m, ArH), 8.12 (2H, d, J = 4.3 Hz, ArH), 8.65 (1H, br s, NH); δ_C (75.5 MHz, DMSO-d_6) 169.78, 167.36, 146.20, 138.35, 135.45, 132.33, 132.10, 130.57, 129.16, 127.52, 126.42, 125.87, 124.97, 124.66, 123.61, 121.50, 120.48, 110.47, 49.32, 46.50, 37.40, 19.83; ESI-MS: Requires 464.15048, Found 464.15096 ($M+H^+$).

N-[[5-[1-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]-3-(trifluoromethyl)benzenesulfonamide (61). White powder; 34% yield; δ_H (300 MHz, MeOH-d_4) 4.47 (2H, s, NCH_2), 4.79 (2H, t, J = 5.1 Hz, NCH_2), 5.00 (2H, t, J = 4.9 Hz, NCH_2), 7.15 (1H, s, HC=CH), 7.25 (1H, s, HC=CH), 7.37 (3H, m, HC=N + ArH), 7.64 (3H, m, ArH), 8.10 (4H, m, ArH); δ_C (75.5 MHz, MeOH-d_4) 172.04, 168.65, 143.45, 142.48, 137.73, 131.80, 131.45, 131.45, 131.32, 130.69, 130.51, 130.20, 129.15, 127.84, 124.96, 123.87, 123.42, 122.29, 110.74, 50.53, 48.19, 39.18; ESI-MS: Requires 518.12222, Found 518.12347 ($M+H^+$).

4-tert-Butyl-N-[[5-[1-(2-imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methyl]benzenesulfonamide (62). White powder; 24% yield; δ_H (300 MHz, MeOH-d_4) 1.31 (9H, s, CH_3), 4.39 (2H, s, NCH_2), 4.67 (2H, t, J = 5.7 Hz, NCH_2), 4.88 (2H, m, NCH_2), 6.84 (1H, s, HC=CH), 6.90 (1H, s, HC=CH), 7.31 (2H, m, HC=N + ArH), 7.44 (3H, m, ArH), 577.74 (3H, m, ArH), 8.08 (1H, d, J = 8.1 Hz, ArH); δ_C (75.5 MHz, DMSO-d_6) 169.78, 167.22, 155.18, 150.96, 145.25, 140.78, 137.44, 129.07, 127.49, 126.38, 125.57, 125.26, 125.26, 124.28, 124.28, 123.60, 121.52, 110.49, 49.64, 47.50, 37.68, 34.26, 31.02, 31.02, 30.351; ESI-MS: Requires 506.19743, Found 506.19612 ($M+H^+$).

N-((5-(2-(2-(1H-Imidazol-1-yl)ethyl)-2H-indazol-3-yl)-1,2,4-oxadiazol-3-yl)methyl)benzenesulfonamide (63). White powder; 28% yield; δ_H (300 MHz, DMSO-d_6) 4.33 (2H, s, NCH_2), 4.58 (2H, t, J = 5.9 Hz, NCH_2CH_2N), 5.23 (2H, t, J = 5.9 Hz, NCH_2), 6.72 (1H, s, HC=CH), 6.94 (1H, s, HC=CH), 7.32 (1H, s, HC=N), 7.47 (5H, m, ArH), 7.84 (3H, m, ArH), 8.12 (1H, d, J = 7.9 Hz, ArH), 8.65 (1H, br s, NH); δ_C (75.5 MHz, DMSO-d_6) 167.072, 167.00, 147.29, 140.55, 137.36, 132.26, 128.92, 128.92, 128.31, 127.11, 126.47, 126.47, 125.73, 122.24, 119.97, 119.42, 118.98, 118.22, 53.25, 45.57, 37.81; ESI-MS: Requires 472.11678 Found 472.11633 ($M+Na^+$).

N-[4-[[5-[2-(2-Imidazol-1-ylethyl)indazol-3-yl]-1,2,4-oxadiazol-3-yl]methylsulfamoyl]phenyl]acetamide (64). δ_H (300 MHz, DMSO-d_6) 10.03 (1H, s, NH), 8.49 (1H, t, J = 6.2 Hz, NH), 7.97 (1H, d, J = 8.2 Hz, ArH), 7.82 (1H, d, J = 8.4 Hz, ArH), 7.67 (2H, d, J = 8.9 Hz, ArH), 7.59 (2H, d, J = 9.0 Hz, ArH), 7.51 – 7.25 (3H, m, ArH), 6.95 (1H, s, ArH), 6.72 (1H, s, ArH), 5.20 (2H, t, J = 6.0 Hz, CH_2), 4.57 (2H, t, J = 6.0 Hz, CH_2), 4.31 (2H, d, J = 5.9 Hz, CH_2), 1.88 (3H, s, CH_3); δ_C (75.5 MHz, DMSO-d_6) 168.62, 166.99, 166.71, 149.64, 147.29, 142.74, 137.36, 133.80, 128.21, 127.71, 127.04, 125.67, 122.22, 119.97, 119.46, 118.87, 118.05, 53.21, 45.65, 37.63, 23.85. LC-MS-EI 507.4 ($M+H^+$).

In Vitro

Preparation of Rat Forebrain Synaptosomes and Homogenates: Experiments were performed using forebrain from Male Wistar rats weighing 175g to 250 g. All efforts were made to reduce the number of animals used, and all experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and the European Community Council Directive of 24 November 1986 (86/609/EEC). After killing of animals

by stunning and decapitation, crude forebrain synaptosomes (heavy and light mitochondrial fraction containing synaptosomes) were prepared as described previously (Garthwaite et al., 2002). Briefly, a known weight of rat forebrain was transferred to a 0.25 M sucrose solution, to a final concentration of 10% (w/v) and the forebrain was homogenized by 8 up-and-down stokes on Braun Potter S motor driven homogenizer set at 900rpm. The synaptosomes were isolated by centrifugation of the homogenate and collection of the appropriate fractions. The isolated synaptasomes were resuspended in assay buffer to make up a 0-25 mg wet weight per mL solution. Homogenates were then prepared by transferring a known weight of the homogenate to a cooled tube containing ice-cold 50 mM HEPES buffer, pH 7.4. The mixture was homogenized using an Ultra-Turrax homogenizer set at maximum speed. The resulting homogenate was centrifuged so the appropriate fraction could be isolated. This fraction could be resuspended in the assay buffer to make up a 0-25 mg wet weight per mL solution.

Binding Studies: Binding of compounds to synaptosomes was measured using a radioligand displacement assay described by Garthwaithe et al., 2002. Assays were carried out using 14 mL polypropylene test tubes to which a range of concentrations of the compounds under test were added. Test compounds were dissolved in DMSO and added to assays such that the maximum concentration of DMSO did not exceed 2% v/v. 12.5 mg (approximately 500 µg of protein) original wet weight of tissue was added to tubes that contained [^{3}H]BW202W92 (concentration measured independently by radioactivity counting), tetrodotoxin (1 µM unless otherwise indicated), assay buffer and the compounds under test, in a final volume of 1 ml. Assays were carried out in buffer that consisted of 50 mM HEPES (adjusted to pH 7.4 with Tris base), 5.5 mM D-glucose, 0.8 mM MgSO4 and either 1 mM KCl and 134 mM choline chloride or 100 mM KCl and 35 mM choline chloride. Samples were mixed and then incubated for 40 min at 25°C. Incubations were terminated by the addition of 5 ml of ice-cold wash buffer consisting of 163 mM choline chloride, 1.8 mM CaCl2 and 0.8 mM MgSO4 in 5 mM HEPES buffer, pH 7.4, followed immediately by vacuum filtration through GF/C glass fiber filters (Whatman, Maidstone, UK) using a Brandel cell harvester (Brandel Inc., Gaithersburg, MD). A further 2-5 ml of ice-cold wash buffer was added to each tube, and the vacuum filtration step was repeated. The GF/C glass fiber filters containing bound [^{3}H]BW202W92 were transferred to minivials, and 4 ml of Picofluor 40 liquid scintillant was added using a Brandel deposit/dispense system. Radioactivity was measured using a liquid scintillation counter (Beckman Coulter, Inc., Fullerton, CA), and cpm was converted directly to dpm via reference to appropriate quench parameters.

Hippocampal Slice Assay. Assays were performed as previously described (Clutterbuck et al., 2009) Briefly, following killing and decapitation of the animal, the forebrain was rapidly dissected and transferred to artificial cerebral spinal fluid (aCSF). Hippocampi were dissected and sliced using a McIlwain tissue chopper. Slices were incubated at 30oC with 95% O2/5% CO2 for 1 hr before medium was replaced by 25 mL of (30 °C) Ca2+-free aCSF containing compounds under test. Following a further 10 min, two to three slices were removed for measurement of ATP and protein by immediate transfer to individual microfuge tubes containing trichloroacetic acid (TCA). Iodoacetate was added to the remaining flasks, and

gassing discontinued. Exactly 11 min later, three to four slices were removed and transferred to microfuge tubes containing TCA (as above) for measurement of ATP and protein.

Measurement of ATP and Protein: Briefly, individual slices were ultrasonicated and the resulting homogenates centrifuged (10,000 rpm for 5 mins at 4 °C). the resulting pellet was resuspended in 5mL 0.1 M KOH by ultrasonication and warmed with gentle agitation at 37°C for 30 min. Concentrations of ATP were measured by mixing with Luciferase reagent (ATPLite from Perkin-Elmer) and measuring subsequent luminescence. Protein concentrations were measured using BCA protein assay (Pierce) with bovine serum albumin as standard. ATP concentrations were expressed as nmol/mg protein and % neuroprotection was calculated by comparison with 1 μM tetrodotoxin.

Na_v Isoform Screening: Patch-clamp experiments against $hNa_v1.1$ to $hNa_v1.8/\beta3$ were performed by Chantest Inc., Cleveland, OH. Briefly, test compounds were evaluated at room temperature using the PatchXpress 7000A (Molecular Devices), an automatic parallel patch-clamp system. Compounds were evaluated at 0.1, 0.3, 1, 3, 10, 30 and 100 μM Concentration, being tested in two to four cells. The duration of each exposure was 5 min. Use-dependence of inhibition on $hNa_v1.1$ and $hNa_v1.8/\beta3$ was determined using a double pulse protocol. A holding potential of -80 mV and prepulse potential of -120 mV were followed by pulse 1-0 mV for 200 ms. An interpulse potential of -80 mV was then applied for 200 ms, followed by a second pulse of 0 mV for 20 ms. The pulse pattern was repeated at 10 s intervals (0.1 Hz), and peak current amplitudes at both test pulses were measured. Percentage use-dependent inhibition (I) was calculated from the equation $I = (1 - P2T/P1T) \times P1C/P2C \times 100$, where P1 and P2 refer to the current amplitudes produced by the first and second pulses, respectively,

RatHepaticMicrosomalStability. Briefly, compounds (1μM) were incubated (n=2) with pooled rat liver microsomes (0.25mg protein/ml) at 37^{o}C for 0 and 40 min before termination of reactions and extraction of compound with acetonitrile containing carbamazepine as analytical internal standard. Samples were centrifuged and the resultant supernatant analysed for disappearance of parent compound by mass spectrometry (LC-MS/MS). The instrument responses (peak heights) of the incubated samples were referenced to the zero time-point samples in order to determine the percentage turnover of compound.

Mass spectrometer parameters.

Parameter	**Setting**
Capillaryvoltage(kV)	3.0
Extractorconevoltage(V)	3
RFlens(V)	0.2
Sourcetemp(^{o}C)	120
Desolvationgastemp(^{o}C)	250
Desolvationgasflow(L/h)	350
Conegasflow(L/h)	100

Chromatographic conditions

Parameter	**Setting**	
Column	ZorbaxSB	C830x4.6mm3µm
Flowrate		1.0ml/min
Injectionvolume		10µL
Mobilephase	A	0.01%Formicacidinwater
	B	0.01%Formicacidinacetonitrile
Gradientprofile	0.0min	5%B
	2.5min	95%B
	4.0min	95%B
	or	
	0.0min	5%B
	1.0min	95%B
	2.0min	95%B
	2.1min	5%B

In Vivo

Animals: Adult (6-8 weeks) Biozzi ABH mice were purchased from Harlan UK Ltd (Bicester, UK) or were bred from stock at Queen Mary University of London. These were maintained on 12h: 12h light: dark cycle and received food and water *ad libitum*. All procedures were approved by the local ethical review processes and were in accordance with UK Animals (Scientific Procedures) Act 1986.

***In vivo* Local lymph node Assay:** Mice (n=3-4/group) received epicutaneous application of 25µl of acetone: olive oil [4:1.AOO] or 25µl of 2.5% oxazolone (OX. Simga Poole, UK) in AOO on day 0 on the dorsum of the ear. On day 3 the draining auricular lymph nodes were removed and a single cell suspension was made. Lymph node cells were counted and 5 x 10^5 cells/well, in triplicate, were cultured overnight at 37°C/5% CO_2 in 200µl of RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum, L-glutamine, sodium-pyruvate and antibiotics as described previously (O'Neill et al. 1992a). T cell proliferation was assessed using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay according to the manufacturer's instructions (Promega, Southhampton UK). Animals received daily intra-peritoneal (i.p.) injections of either ethanol:cremophor: phosphate buffered saline [1:1:18] or CFM6104 from day -1-2

Induction of chronic-relapsing EAE: Mice received subcutaneous injections in the flank of 1mg freeze-dried mouse spinal cord homogenate (SCH) in Freund's adjuvant supplemented with 60µg *Mycobacterium tuberculosis* H37Ra and *M. butyricum* on day 0 and 7, as previously described (Baker et al. 1990). Following the development of the initial paralytic disease and subsequent remission a relapse was induced by a further injection of SCH in

Freunds adjuvant on day 27 (O'Neill et al. 1992b). Animals were monitored and weighed daily to assess the development of relapsing-remitting paralysis. *Clinical scores* were graded as 0 = Normal; 1 = Limp tail, 2 = impaired righting reflex, 3 = hindlimb paralysis and 4 = complete hindlimb paralysis, 5 = moribund/death. Signs of reduced severity were scored at 0.5 less than the indicated grade as described previously (Baker et al. 1990, O'Neill et al. 1992a). A clinical relapse was associated with an increase in neurological disease and weight loss (>5%) as described previously (Baker et al. 1990). *RotaRod:* Motor control and co-ordination was assessed on an accelerating (4–40 rpm, accelerating at 6rpm/25s) RotaRod (ENV-575M. Med Associates Inc, St. Albans, VT, USA). Activity was assessed during the remission phases of the disease, over a maximum 5 minute observation period. The trial was terminated when the mouse either fell from the RotaRod spindle or if the mouse failed to tolerate the revolving drum shown by holding onto the RotaRod spindle rod for two consecutive turns. Following the develop of the initial acute phase paralytic attack and remission animals were randomized within cages, based on clinical score, to receive either an intra-peritoneal (i.p.) injection of either vehicle or CFM6104. This was administered just before the anticipated onset of relapsing disease from day 33 post-induction (p.i.) onwards.

Neurofilament ELISA. An enzyme linked immunosorbent assay for heavy chain neurofilament was performed as previously described (Jackson et al. 2005). Briefly whole spinal cord samples were homogenized with a glass homogenizer, centrifuged (20,000g) and the supernatant was collected for analysis. A 96 well plate was coated overnight at 4°C with capture antibody (SMI-35 anti-neurofilament H. Covance Inc. Cambridge Bioscience, Cambridge, UK). Following one wash in wash buffer (11 mM barbital, 63 mM sodium barbital, 1.2 mM EDTA, Sigma), non-specific binding was blocked by incubation with 5% bovine serum albumin in wash buffer for 1 hour at room temperature. Following a wash step, samples and standards (Bovine neurofilament heavy chain. Sigma, Poole, UK) were diluted in wash buffer with 1% bovine serum albumin and incubated on the plate for 2 hours at room temperature. Following 3 wash steps, the horseradish peroxidase conjugated, detector antibody was applied (rabbit anti-NF200, Sigma) and incubated for a further hour at room temperature. Following a final 3 washes, tetramethylbenzidine substrate was applied and colour production measured on a Synergy HT plate reader at 405nm with a reference at $720nm^2$.

Statistical analysis: The data is presented as mean ± standard error of the mean (SEM). Differences in clinical scores between groups were assessed using non-parametric, Mann Whitney U statistics. The parametric data was assessed using t tests, incorporating tests for equality of variance using Sigmastat software (Systat Software, Inc., San Jose, USA).

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