# Supporting Information

## Discovery of the 1st Orally Available, Selective $K_{Na}1.1$ Inhibitor: *In Vitro* and *In Vivo* Activity of an Oxadiazole Series

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Number of pages: 17 (including cover)

Number of figures: 6

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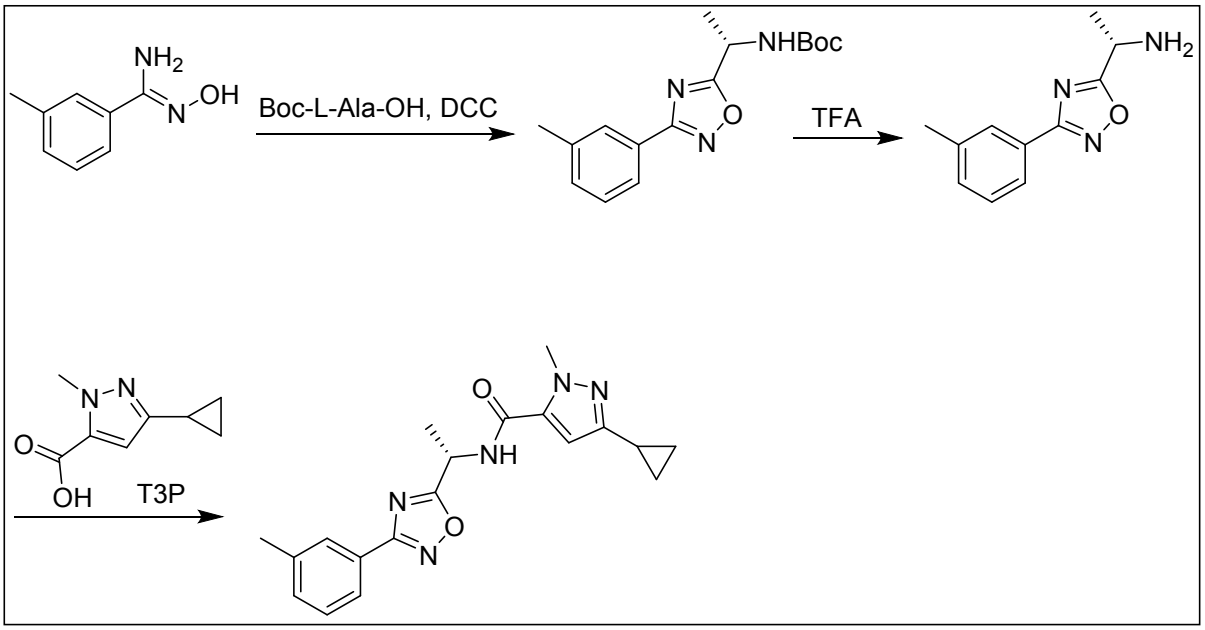
**Figure S1. Synthesis of Compound 3**




**tert-Butyl (S)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate**

To a solution of N'-hydroxy-3-methylbenzimidamide (1.0 g, 6.25 mmol) in 1,4-dioxane (60 mL) was added (2S)-2-(tert-butoxycarbonylamino)propanoic acid (1.37 g, 7.22 mmol) followed by DCC (1.51 g, 7.32 mmol). The reaction mixture stirred at 100 ˚C for 16 h. The reaction was concentrated to dryness and the residue was taken up in EtOAc and the organics washed with water and then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness. The crude was then purified by flash column chromatography. The desired fractions were concentrated to dryness in vacuum to give tert-butyl (S)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (0.80 g, 2.62 mmol, 39.4% yield, 99.6% purity). LCMS: $C_{16}H_{20}N_3O_3$ $[M-H]^-$: calculated 302.1, found 302.1; Chiral HPLC purity (*ee*): 100%.

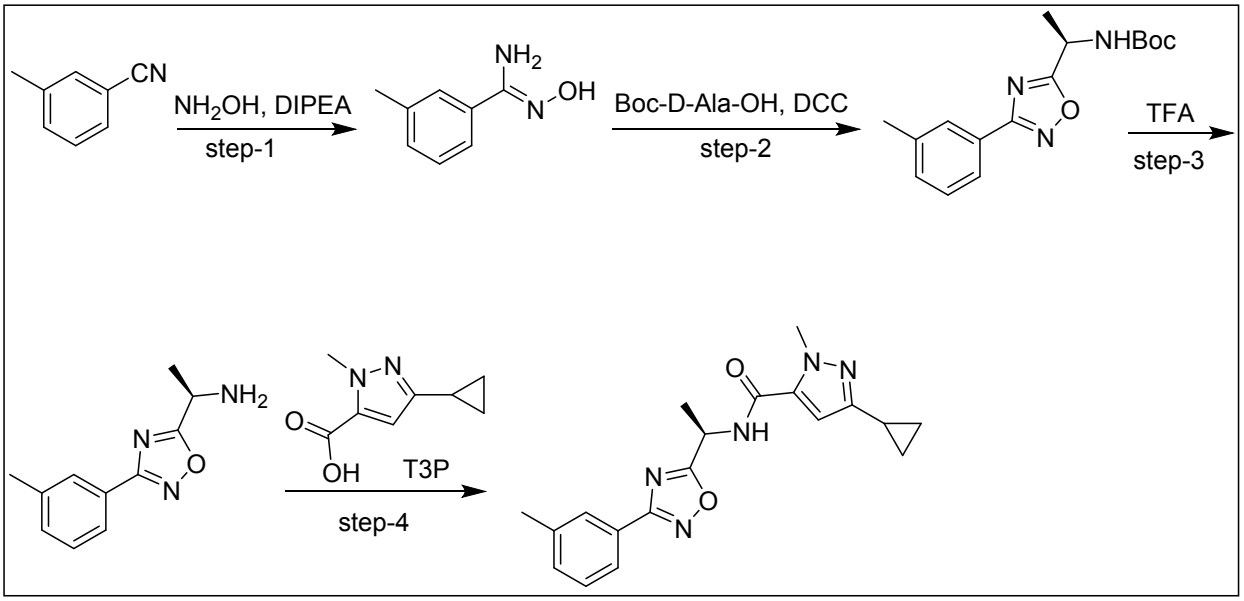
**(S)-1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine**

To a stirred Solution of TFA (2.13 mL, 27.8 mmol) in DCM (2.4 mL) at 0 ˚C tert-butyl (S)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (400 mg, 1.32 mmol) was added and stirred for 8 h at RT. The reaction mixture was concentrated and then treated with saturated $NaHCO_3$ solution and taken up in EtOAc and the organics washed with water then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness to give (S)-1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine (200 mg, 0.98 mmol, 74% yield, 99.7% purity). LCMS: $C_{11}H_{14}N_3O$ $[M+H]^+$: calculated 204.1, found 204.1; Chiral HPLC purity (*ee*): 100%.

**(S)-3-Cyclopropyl-1-methyl-N-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)-1H-pyrazole-5-carboxamide**

To a stirred solution of 3-cyclopropyl-1-methyl-1H-pyrazole-5-carboxylic acid (179 mg, 1.08 mmol) in THF (10 mL), T3P (1.17 mL, 1.97 mmol) and TEA (0.41 mL, 20.52 mmol) were added. The reaction mixture was stirred at RT for 10 min and (S)-1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl) ethan-1-amine (200 mg, 0.98 mmol) was added. The reaction mixture was stirred for 16 h at RT. The reaction mixture was concentrated to dryness and the residue was taken up in EtOAc and the organics washed with water then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness. The crude was then purified by flash column chromatography and the desired fractions were concentrated to dryness in vacuum to give (S)-3-cyclopropyl-1-methyl-N-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)-1H-pyrazole-5-carboxamide (80 mg, 0.22 mmol, 23.0% yield). HPLC purity: 99.76%; LCMS $[M+H]^+$: 352.3; Chiral HPLC purity (*ee*): 100%; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta_H$ 7.91-7.88 (m, 2H), 7.42-7.34 (m, 2H), 6.58 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 1H), 5.62-5.58 (m, 1H), 4.12 (s, 3H), 2.45 (s, 3H), 1.97-1.93 (m, 1H), 1.75 (d, *J* = 7.2 Hz, 3H), 0.99-0.94 (m, 2H), 0.78-0.74 (m, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 179.1, 168.5, 159.2, 153.4, 138.7, 134.7, 132.2, 128.8, 128.0, 126.2, 124.7, 103.1, 42.9, 39.0, 21.4, 19.9, 8.8, 8.0. $C_{19}H_{22}N_5O_2^+$ $[M+H]^+$ 352.1768, found 352.1766.

**Figure S2. Synthesis of Compound 4**




**N'-Hydroxy-3-methylbenzimidamide**

To a solution of 3-methylbenzonitrile (10 g, 85.36 mmol) in ethanol (200 mL) was added hydroxylamine hydrochloride (17.8 g, 256.08 mmol) followed by DIPEA (8.63 mL, 85.36 mmol). Resulting reaction mixture was stirred at 70 ˚C for 16 h. After completion of the reaction, ethanol was removed under vacuum and diluted with water and extracted with ethyl acetate and concentrated under reduced pressure to give N'-hydroxy-3-methylbenzimidamide (10 g, 62.5 mmol, 73.3% yield, 94% purity). LCMS: $C_8H_{11}N_2O$ $[M+H]^+$: calculated 151.1, found 151.1.

***tert*-Butyl (R)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate**

To a solution of N'-hydroxy-3-methylbenzimidamide (2.13 g, 13.32 mmol) in 1,4-dioxane (60 mL) was added (2R)-2-(tert-butoxycarbonylamino)propanoic acid (2.52 g, 13.32 mmol) followed by DCC (3.02 g, 14.65 mmol). The reaction mixture stirred at 100 ˚C for 16 h. The reaction was concentrated to dryness and the residue was taken up in EtOAc and the organics washed with water and then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness. The crude was then purified by flash column chromatography. The desired fractions were concentrated to dryness in vacuum to give tert-butyl (R)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (1.2 g, 3.8 mmol, 28.6% yield, 96.3 % purity). LCMS: $C_{16}H_{20}N_3O_3$ $[M-H]^-$: calculated 302.1, found 302.1; Chiral HPLC purity (*ee*): 99%.

**(R)-1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine**

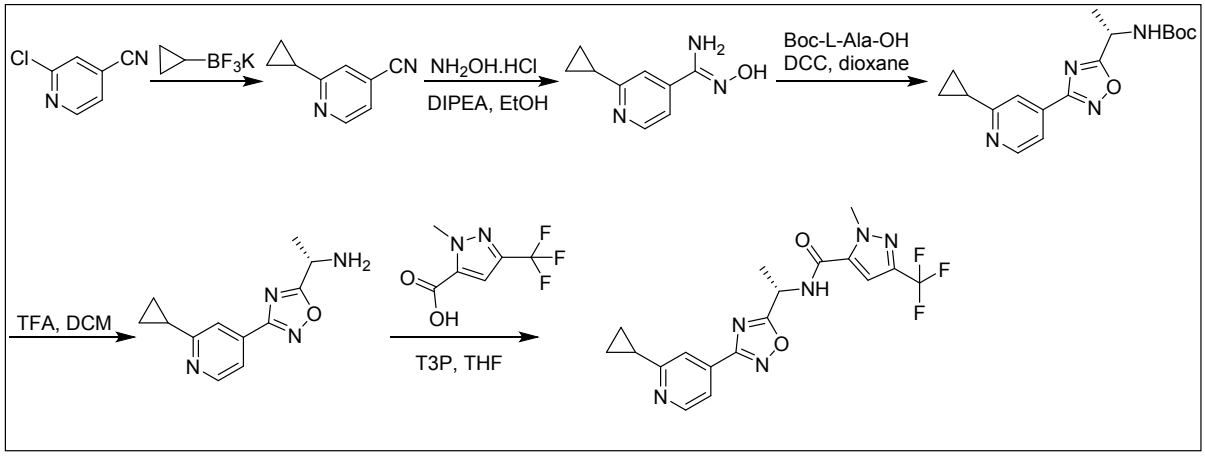
To a stirred solution of TFA (1.5 mL, 20.48 mmol) in DCM (2.5 mL) at 0 ˚C, tert-butyl (R)-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (**3**, 500 mg, 1.65 mmol) was added and stirred for 8 h at RT. The reaction mixture was concentrated and then treated with saturated $NaHCO_3$ solution and taken up in EtOAc and the organics washed with water then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness to give (1R)-1-[3-(m-tolyl)-1,2,4-oxadiazol-5-yl]ethanamine (200 mg, 0.92 mmol, 56.0% yield, 93.9% purity). LCMS: $C_{11}H_{14}N_3O$ $[M+H]^+$: calculated 204.1, found 204.1; Chiral HPLC purity (*ee*): 99%.

**(R)-3-cyclopropyl-1-methyl-N-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)-1H-pyrazole-5-carboxamide**

To a stirred solution of 3-cyclopropyl-1-methyl-1H-pyrazole-5-carboxylic acid (179 mg, 1.08 mmol), T3P (1.17 mL, 1.97 mmol) and TEA (0.41 mL, 2.95 mmol) were added. The reaction mixture was stirred at RT for 10 min and (1R)-1-[3-(m-tolyl)-1,2,4-oxadiazol-5-yl]ethanamine (200 mg, 0.92 mmol) was added. The reaction mixture was stirred for 16 h at RT. The reaction mixture was concentrated to dryness and the residue was taken up in EtOAc and the organics

washed with water then saturated brine solution. The organics were then separated and dried ($Na_2SO_4$) before concentration to dryness. The crude was then purified by flash column chromatography and the desired fractions were concentrated to dryness in vacuum to give (R)-3-cyclopropyl-1-methyl-N-(1-(3-(m-tolyl)-1,2,4-oxadiazol-5-yl)ethyl)-1H-pyrazole-5-carboxamide (105 mg, 0.29 mmol, 30.1% yield). HPLC purity: 99.26%; LCMS $[M+H]^+$: 352.1; Chiral HPLC purity (*ee*): 99%; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta_H$ 7.91-7.88 (m, 2H), 7.42-7.34 (m, 2H), 6.59 (d, *J* = 8.0 Hz, 1H), 6.32 (s, 1H), 5.64-5.57 (m, 1H), 4.12 (s, 3H), 2.45 (s, 3H), 1.97-1.92 (m, 1H), 1.75 (d, *J* = 7.2 Hz, 3H), 0.99-0.94 (m, 2H), 0.78-0.74 (m, 2H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta_C$ 178.1, 167.4, 158.2, 152.4, 137.7, 133.6, 131.2, 127.8, 126.9, 125.1, 123.6, 102.1, 41.8, 37.9, 20.3, 18.9, 7.8, 6.9, 0.1. $C_{19}H_{22}N_5O_2^+$ $[M+H]^+$ 352.1768, found 352.1766.

**Figure S3. Synthesis of Compound 27**




**2-Cyclopropylisonicotinonitrile**

To a solution of 2-chloropyridine-4-carbonitrile (2.0 g, 14.4 mmol) in 1,4-dioxane (25 mL) was added potassium cyclopropyltrifluoroborate (6.41 g, 43.3 mmol) followed by $K_2CO_3$ (7.98 g, 57.7 mmol) and RuPhos (1.35 g, 2.89 mmol). The resulting mixture was degassed with $N_2$ gas for 10 min and $Pd(OAc)_2$ (324 mg, 1.44 mmol) was added. The mixture was stirred at 100 ºC for 1 h. The reaction mixture was cooled to room temperature and filtered through celite. The filtrate was concentrated under reduced pressure and the crude was purified by column chromatography on silica gel with 15% EtOAc/petether to afford 2-cyclopropylisonicotinonitrile (1.1 g, 7.6 mmol, 50% yield) as an off-white solid.

**(Z)-2-Cyclopropyl-N'-hydroxyisonicotinimidamide**

To a solution of 2-cyclopropylisonicotinonitrile (450 mg, 3.1 mmol) in ethanol (15.0 mL) was added hydroxylamine hydrochloride (312 mg, 4.4 mmol) followed by DIPEA (1.49 mL, 8.99 mmol) at room temperature. The reaction mixture was heated at 80 ºC for 5 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude was treated with water (30 mL) followed by saturated sodium bicarbonate solution (20 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layer was washed with brine (10 mL), dried over $Na_2SO_4$ and concentrated to afford (Z)-2-cyclopropyl-N'-hydroxyisonicotinimidamide (420 mg) as an off-white solid. The material was used for the next step without further purification.

***tert*-Butyl (S)-(1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate**

To a solution of (2S)-2-(tert-butoxycarbonylamino)propanoic acid (0.44 g, 2.31 mmol) in 1,4-dioxane (10.0 mL) was added (Z)-2-cyclopropyl-N'-hydroxyisonicotinimidamide (0.41 g, 2.31 mmol) followed by DCC (0.52 g, 2.55 mmol). The resulting mixture was stirred at 100 ºC for 16 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The mixture was treated with water (30 mL) and extracted with ethyl acetate (2 x 20 mL), separated then concentrated. The crude was purified by column chromatography on silica gel with 15% ethyl acetate/petether to afford tert-butyl (S)-(1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (570 mg, 1.72 mmol, 74% yield) as an off-white solid. LCMS: $C_{17}H_{22}N_4O_3$ $[M+H]^+$: calculated 331.2, found 331.2.

**(S)-1-(3-(2-Cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethan-1-amine (5)**

To a solution of tert-butyl (S)-(1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)carbamate (410 mg, 1.24 mmol) in DCM (5.0 mL) was added TFA (1.36 mL) at 0 ºC. The reaction mixture was slowly brought to room temperature and stirred for 3 h. The mixture was concentrated under reduced pressure and treated with ice water (20 mL). The mixture was treated with 10% aqueous $NaHCO_3$ solution (5 mL) and extracted with EtOAc (2 x 25 mL). The organic layer was washed with brine (20 mL), dried over anhydrous $Na_2SO_4$ and concentrated to afford (S)-1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethan-1-amine (240 mg). The compound was used for the next step without further purification.

**(S)-N-(1-(3-(2-Cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide**

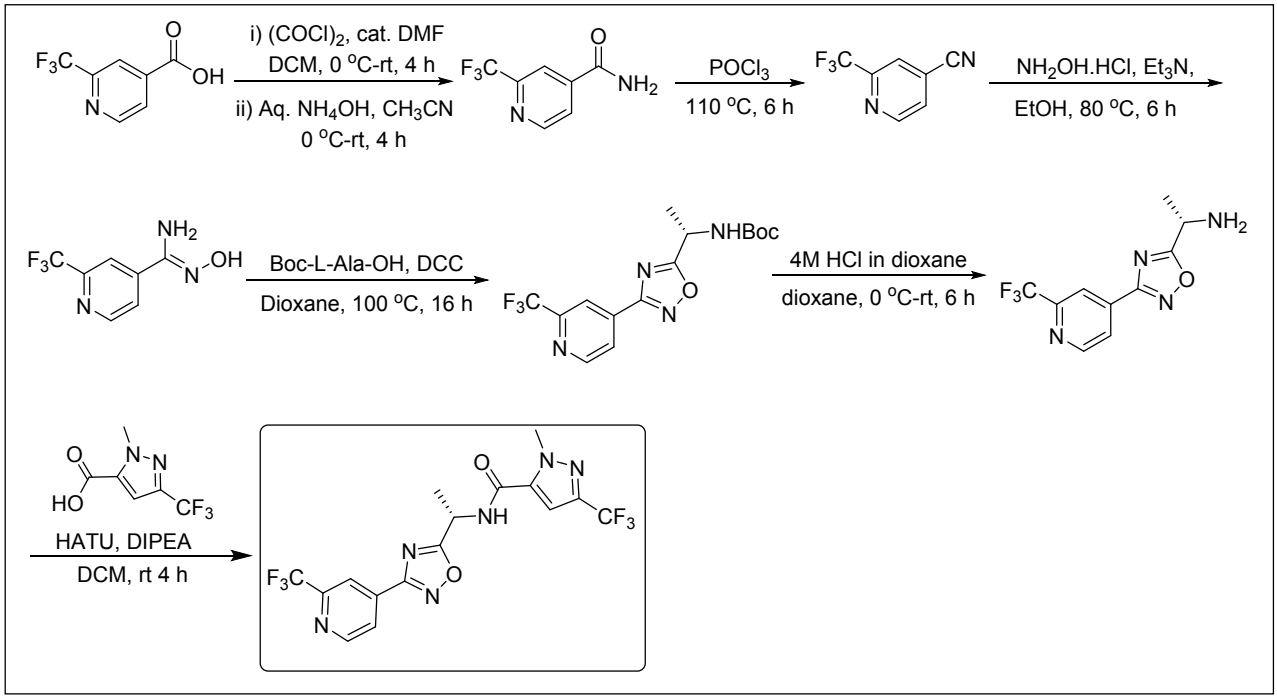
To a solution of (S)-1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethan-1-amine (240 mg, 1.04 mmol) in THF (8.0 mL) was added 2-methyl-5-(trifluoromethyl)pyrazole-3-carboxylic acid (202 mg, 1.04 mmol) followed by $Et_3N$ (0.43 mL, 3.13 mmol) and T3P (50% in EtOAc, 1.86 mL,

3.13 mmol). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was treated with water (30 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layer was washed with brine (20 mL), dried over $Na_2SO_4$ and concentrated. The crude was purified by column chromatography on silica get with 60% EtOAc/petether to afford (S)-N-(1-(3-(2-cyclopropylpyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)-1-methyl-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide (216 mg, 0.53 mmol, 51% yield) as off-white solid.

**HPLC**: Rt 3.48 min, 97.38%
Column: X-Bridge C8 (50 X 4.6) mm, 3.5 µm
Mobile phase: A: 0.1% TFA in water, B: 0.1% TFA in ACN; Flow Rate: 2.0 mL/min
**LCMS**: 407.1 (M+H), Rt 1.77 min, 99.78%
Column: ZORBAX XDB C-18 (50 X 4.6 mm), 3.5 µm
Mobile Phase: A: 0.1% TFA in water:ACN (95:5), B: 0.1% TFA in ACN; Flow Rate:1.5 mL/min
**Chiral method:** Rt 1.42 min, SFC column: YMC Cellulose-SB; mobile phase: 60:40 (A: B), A = liquid $CO_2$, B = 0.5% isopropyl amine in methanol; flow rate: 3.0 mL/min; wave length: 220 nm.
**$^1$H NMR (400 MHz, $CDCl_3$):** $\delta_H$ 8.61 (d, *J* = 4.8 Hz, 1H), 7.79 (s, 1H), 7.68 (dd, *J* = 1.6 Hz and 5.2 Hz, 1H), 6.95 (s, 1H), 6.70 (d, *J* = 8.0 Hz, 1H), 5.67-5.60 (m, 1H), 4.25 (s, 3H), 2.20-2.13 (m, 1H), 1.80 (d, J = 7.2 Hz, 3H), 1.14-1.10 (m, 4H).
**$^{13}$C NMR** (100 MHz, $CDCl_3$) $\delta_C$ = 178.6, 166.1, 163.3, 157.2, 148.6, 140.1, 139.7, 134.5, 132.8, 120.9, 117.8, 116.9, 104.2, 42.0, 39.2, 18.6, 16.2, 9.452, 0.01.

$C_{18}H_{18}F_3N_6O_2^+$ $[M+H]^+$407.1438, found 407.1449.

**Figure S4. Synthesis of Compound 31**




**2-(trifluoromethyl)isonicotinamide**

To a stirred solution of 2-(trifluoromethyl)pyridine-4-carboxylic acid **1** (15.0 g, 78.49 mmol) in DCM (150 mL) at 0 ºC was added oxalyl chloride (10.46 g, 82.41 mmol) and catalytic amount of DMF (2 mL). The reaction mixture was warmed to room temperature and stirred for 4 h. The reaction mixture was concentrated under reduced pressure resulting in the corresponding acid chloride. To a two neck 500 mL round bottom flask at 0 °C was added aqueous $NH_4OH$ (2.0 mL) followed by solution of acid chloride in acetonitrile (20 mL) dropwise. The reaction mixture was further stirred at room temperature for 4 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure and crude residue obtained was stirred with ethyl acetate (150 mL). The organic layer was washed with water (2 x 50 mL), followed by brine (50 mL), dried over $Na_2SO_4$ and concentrated to afford 2-(trifluoromethyl)isonicotinamide (14.0 g, 69.75 mmol, 88.8%). The compound was used for the next step without further purification.

**2-(Trifluoromethyl)pyridine-4-carbonitrile**

$POCl_3$ (80 mL, 858.28 mmol) was added dropwise to 2-(trifluoromethyl)isonicotinamide (8.00 g, 42.08 mmol) at 0 ºC. The reaction mixture heated at 110 ºC for 6 h. The reaction mixture was cooled to room temperature and concentrated upto dryness and poured over ice cold water and neutralized with 50% NaOH solution (50 mL) and diluted with ethyl acetate (200 mL). The organic layer was washed with water (2 x 50 mL) followed by brine (50 mL), dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure resulting in the crude compound. The crude

compound was purified by column chromatography on silica gel eluting with 0-10% ethyl acetate in hexane to afford 2-(Trifluoromethyl)pyridine-4-carbonitrile (8.00 g, 46.48 mmol) as a brown liquid.

**LCMS**: 173.1 (M+H), Rt 1.84 min

Column: ZORBAX XDB C-18 (50 X 4.6 mm), 3.5 µm

Mobile Phase: A: 0.1% HCOOH in water:ACN (95:5), B: ACN; Flow Rate:1.5 mL/min

***N*'-Hydroxy-2-(trifluoromethyl)pyridine-4-carboxamidine**

To a stirred solution of 2-(Trifluoromethyl)pyridine-4-carbonitrile (11.0 g, 63.91 mmol) in ethanol (100 mL) was added hydroxylamine hydrochloride (6.66 g, 95.87 mmol) and triethyl amine (17.7 mL, 127.83 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was heated at 80 ºC for 6 h. The reaction mixture was concentrated under reduced pressure and crude residue was diluted with ethyl acetate (100 mL) and water (50 mL). The organic layer was separated and washed with water (2 x 50 mL) followed by brine (50 mL), dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to afford *N*'-Hydroxy-2-(trifluoromethyl)pyridine-4-carboxamidine (12.0 g, 53.10 mmol, 83.1%) as an off-white solid which was used in the next step without further purification.

***tert*-Butyl (*S*)-(1-(3-(2-(trifluoromethyl)pyridin-4-yl)-1,2,4-oxadiazol-5-yl) ethyl)carbamate**

To a stirred solution of *N*'-Hydroxy-2-(trifluoromethyl)pyridine-4-carboxamidine (8.00 g, 38.99 mmol) in 1,4-dioxane (100 mL) was added (2S)-2-(tert-butoxycarbonylamino)propanoic acid (8.12 g, 42.90 mmol) and DCC (8.84 g, 42.90 mmol) at room temperature. The reaction mixture was further heated at 100 ºC for 16 h. The reaction mixture was concentrated under reduced pressure and crude residue was diluted with ethyl acetate (200 mL) and water (50 mL). The organic layer was separated and washed with water (2 x 50 mL) followed by brine (50 mL), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure resulting in the crude compound. The crude compound was purified by flash column chromatography eluting with 0-30% ethyl acetate in hexane to afford *tert*-Butyl (*S*)-(1-(3-(2-(trifluoromethyl)pyridin-4-yl)-1,2,4-oxadiazol-5-yl) ethyl)carbamate (13.5 g, 27.37 mmol, 70.1% yield) as an off white solid.

**LCMS**: 359.2 (M+H), Rt 2.42 min

Column: ZORBAX XDB C-18 (50 X 4.6 mm), 3.5 µm

Mobile Phase: A: 0.1% HCOOH in water:ACN (95:5), B: ACN; Flow Rate:1.5 mL/min

**(1*S*)-1-[3-[2-(trifluoromethyl)-4-pyridyl]-1,2,4-oxadiazol-5-yl]ethanamine**

To a stirred solution of *tert*-Butyl (*S*)-(1-(3-(2-(trifluoromethyl)pyridin-4-yl)-1,2,4-oxadiazol-5-yl) ethyl)carbamate (13.5 g, 37.68 mmol) in dioxane (20 mL) at 0 $^{o}$C was added 4M HCl in dioxane (100 mL). The reaction mixture was slowly warmed to room temperature and stirred for 6 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure upto dryness. Crude residue obtained was purified by trituration with diethyl ether to afford (1*S*)-1-[3-[2-(trifluoromethyl)-4-pyridyl]-1,2,4-oxadiazol-5-yl]ethanamine
(11.0 g, 35.04 mmol, 93.01%) as an off white solid.

**(*S*)-1-methyl-3-(trifluoromethyl)-*N*-(1-(3-(2-(trifluoromethyl)pyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)-1*H*-pyrazole-5-carboxamide**

To a stirred solution of (1*S*)-1-[3-[2-(trifluoromethyl)-4-pyridyl]-1,2,4-oxadiazol-5-yl]ethanamine (5.00 g, 16.01 mmol) and 2-methyl-5-(trifluoromethyl)pyrazole-3-carboxylic acid (4.28 g, 22.06 mmol) in DCM (100 mL) was added DIPEA (8.87 mL, 50.91 mmol) followed by HATU (9.68 g, 25.45 mmol) at room temperature and stirred for 4 h. After completion of reaction, the reaction mixture was diluted with water (30 mL) and extracted with DCM (2 x 30 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure resulting in the crude compound. The crude compound was purified by 100-200 mesh size silica gel column chromatography eluting with 0-50% ethyl acetate in n-hexane to afford (*S*)-1-methyl-3-(trifluoromethyl)-*N*-(1-(3-(2-(trifluoromethyl)pyridin-4-yl)-1,2,4-oxadiazol-5-yl)ethyl)-1*H*-pyrazole-5-carboxamide (4.00 g, 9.145 mmol, 54% yield) as an off-white solid.

**LCMS**: 434.90 (M+H), Rt 2.162 min, 98.93%

Method:- LCMS_X-Select (Formic acid)

Column: X-Select CSH C18 (3.0*50) mm 2.5 µ; Mobile Phase: A: 0.05% Formic acid in water: ACN (95:5); B: 0.05% Formic acid in ACN; Inj Volume: 2.0µL, Column oven temperature: 50 C; Flow Rate: 1.2.mL/minute; Gradient program: 0% B to 98 % B in 2.0 minute, hold till 3.0 min, at 3.2 min B conc is 0 % up to 4.0 min

**HPLC**: Rt 9.147 min, 99.37%

Column: X-Bridge CSH C18 (4.6* 150) mm, 3.5 µ;

Mobile phase: A: 0.1% Formic acid in water, B: Acetonitrile (95:05)

B: Acetonitrile

Flow Rate: 1.0 mL/min; Gradient program: Time (min)/ BConc: 0.01/5, 1.0/5, 8.0/100, 12.0/100, 14.0/5, 18.0/5

**CHIRAL HPLC**: Rt 11.40 min, 96.50%

Column: PHENOMENEX CELLULOSE-1 (250 *4.6 mm, 5 µ), Mobile Phase: A) *n*-Hexane+0.1% TFA, B) Iso-propyl-alcohol; Flow rate: 1.0 mL/min; Isocratic: 10%B.

**Q-NMR =** >98% purity

**$^1$H NMR (400 MHz, DMSO-$d_6$):** δ 9.49 (d, *J*=6.36 Hz, 1H), 9.02 (d, *J*=3.91 Hz, 1H), 8.29 (br. s, 2H), 7.45 (br. s, 1H), 5.43-5.56 (m, 1H), 4.13 (br. s, 3H), 1.70 (d, *J*=6.85 Hz, 3H).

**$^1$H NMR (400 MHz, DMSO-$d_6$; $D_2O$ exchange):** 8.97 (d, *J*=3.91 Hz, 1H), 8.27 (br. s, 1H), 8.24 (d, *J*=4.40 Hz, 1H), 7.38 (br. s, 1H), 5.44 (d, *J*=6.85 Hz, 1H), 4.08 (br. s, 3H), 1.67 (d, *J*=6.85 Hz, 3H)

**$^{13}$C NMR (DMSO):** 181.4, 165.6, 158.1, 151.9, 136.0, 135.5, 124.6, 117.6, 106.8, 42.6, 39.5, 17.8

$C_{16}H_{13}F_6N_6O_2$ $[M+H]^+$435.0999, found 435.1003.

**Experimental Animals**

All experimental procedure in this report were conducted in accordance with the Prevention of Cruelty to Animals Act 1986, under the guidelines of the NHMRC Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia and were approved by the Florey Neuroscience Institute Animal Ethics Committee (AEC 18-126 FINMH).

**Patch-clamp recordings from brain slices**

*Brain Slice Preparation*

Mice (p16-p30) were anesthetized with 2% isoflurane and decapitated. The brain was quickly removed and placed into an iced slurry of brain slice cutting solution composed of (in mM): Choline chloride 125, KCl 2.5, $NaH_2PO_4$ 1.25, $NaHCO_3$ 26, D-glucose 20, $CaCl_2.2H_2O$ 0.4, $MgCl_2.6H_2O$ 6, pH 7.4 maintained by continuous bubbling with carbogen (95% O2 – 5% O2). Three hundred micrometer coronal hippocampal slices were cut on a vibratome (VT1200; Leica) for whole-cell patch-clamp experiments. Prior to patching slices were incubated for a minimum of 1 hour at room temperature in the extracellular recording solution, composed of (in mM): NaCl 125, KCl 2.5, $NaH_2PO_4$ 1.25, $NaHCO_3$ 26, D-glucose 10, $CaCl_2.2H_2O$ 2, $MgCl_2.6H_2O$ 2, pH 7.4 via continuous bubbling with carbogen.

*Current clamp electrophysiology recordings*

Brain slices were transferred to a submerged recording chamber on an upright microscope (Slicescope Pro 1000; Scientifica) and were perfused (2 ml/min) with the external aCSF recording solution (Table 3) at 32°C. CA1 pyramidal neurons were identified visually in the *stratum pyramidale* of the CA1 region of the hippocampus, using infrared-oblique illumination microscopy with a 40x water-immersion objective (Olympus) using a camera (Dage IR-2000; Dage). Cell identify was also confirmed using action potential firing characteristics, where action potentials were accommodating at high current injections and had a wide action potential half-width. Patch-clamp recordings were made using a micromanipulator (MPC-200; Sutter) and Axon Multiclamp 700B patch-clamp amplifier (MDS). Data were acquired using pClamp software (v10; MDS) using a sampling rate of 50 kHz and low pass Bessel filtered at 10 kHz (Digidata 1550b; Axon). Patch pipettes (3-7 MΩ; GC150F-7.5; Harvard Instruments) pulled using a Flaming/brown micropipette puller (Model P-1000; Sutter) were filled with intracellular recording solution consisting of (in mM): K gluconate 125, KCl 5, $MgCl_2.6H_2O$ 2, HEPES 10, ATP-Mg 4, GTP-Na 0.3, Phosphocreatine 10, EGTA 0.1 and biocytin 0.2%, pH 7.2 with KOH and 292 mOsm.

**Experimental protocols and analysis**

All studies were performed using whole-cell current clamp recording mode.

*Assay – Intrinsic Excitability (Evoked Action Potentials)*

Once whole-cell configuration was obtained for 2 min, a holding current was injected to maintain a membrane potential of approximately -70 mV. Current steps (injected current of between -60 to 340 pA in 20 pA steps, 400 ms duration) were applied in current clamp mode. The amplitude of current injections where relative to the holding current. A test pulse (-5 pA amplitude, 50 ms duration) was applied 650 ms after the termination of the main current step. The inter sweep interval was 5 sec (0.2Hz). To be included in the study a cell had to have an access resistance of less than 20 MΩ and a holding current of less than -200 pA. Once baseline action potential firing was determined in the presence of the extracellular aCSF recording solution (baseline) the compound (1 μM or 10 μM) was washed onto the slice for 5 min before repeating the action potential generating protocol.

*Data analysis and statistical analysis*

Data were analyzed using Axograph X software. Individual action potentials were identified using a +50 mV amplitude threshold relative to the pre-event baseline. The number of action potentials generated in response to each current injection was used to create an input-output relationship for each cell. The average number of action potentials evoked for each current injection was then calculated. For statistical comparisons between groups, the total number of action potentials fired per cell was calculated, averaged for the group. Statistical analysis was performed using GraphPad Prism software (v8). Paired two-tailed Student's *t*-tests were used to test the effect of compound on action potential firing compared to baseline. In all cases the significance for analysis was set as an alpha value of 0.05.

**In-Vitro Pharmacology**

Study of Compound 31

The purpose of the study was to test compound 31 in binding assays. Compound 31 was tested at 1.0E-05 M. Compound binding was calculated as a % inhibition of the binding of a radioactively labeled ligand specific for each target. Results showing an inhibition or stimulation higher than 50% are considered to represent significant effects of the test compounds. Such effects were observed here are shown in the below histograms.

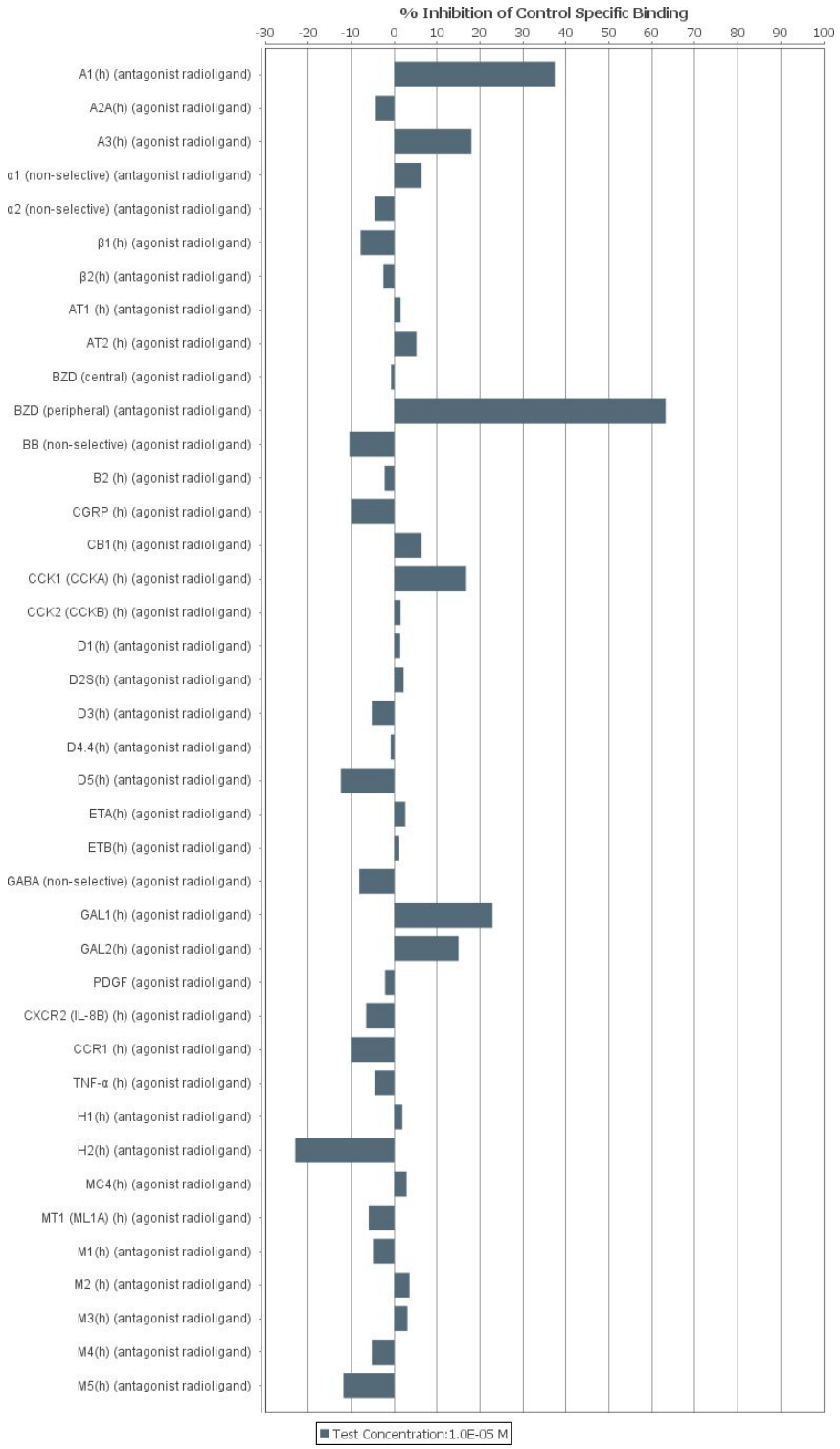



**Figure S5. Histogram for Compound 31 (1/2)**

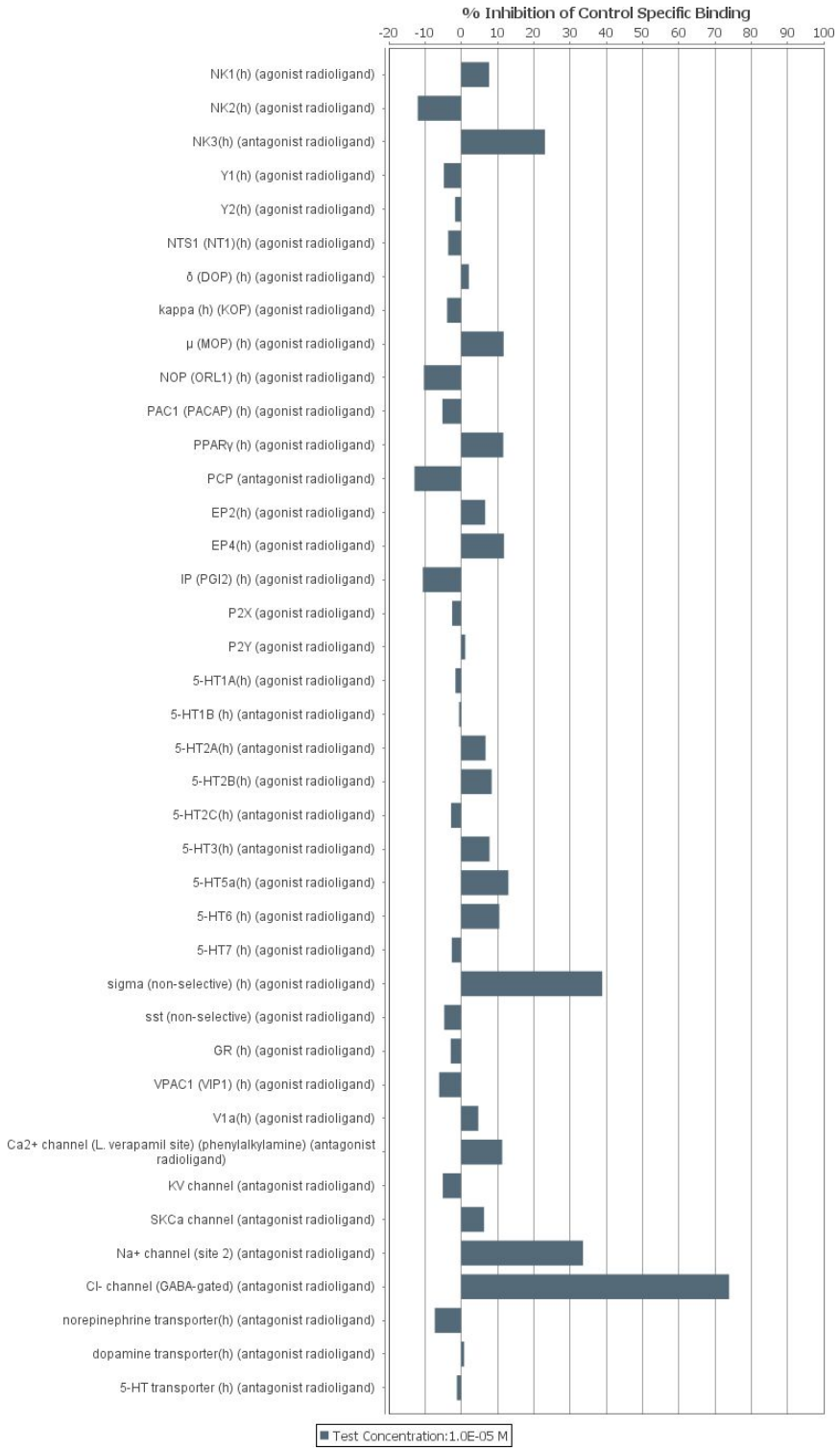



**Figure S6. Histogram for Compound 31 [2/2]**

Further characterization of compound **31** included an assessment of potential secondary activity across a panel of 80 targets at 10μM compound concentration, using binding displacement

assays. Only two hits showed >50% activity: translocator protein (TSPO) (63% displacement) and $GABA_A$ $Cl^-$ channel (74% displacement).