## Supplementary material

## Experimental Section

## Chemistry

### Synthesis of 4-[(2*R*)-4-tert-butylpiperazine-2-carbonyl]-*N*-(5-chloro-4-methyl-1,3-thiazol-2-yl)piperazine-1-carboxamide (2b)

To a solution of tert-butyl (2*R*)-4-tert-butyl-2-(piperazine-1-carbonyl)piperazine-1-carboxylate (330 mg) in tetrahydrofuran (THF) (10 mL) was added triethylamine (195 μL) followed by phenyl *N*-(5-chloro-4-methyl-1,3-thiazol-2-yl)carbamate (330 mg) and the resulting mixture was heated in a microwave oven (Biotage initiator*)* at 60 °C for 1.5 hours. The mixture was concentrated and the resulting product was dissolved in $CH_2Cl_2$ (20 mL), to which trifluoroacetic acid (TFA) (10 mL) was added and the resulting mixture was stirred at room temperature for 1.5 hours. The reaction mixture was poured onto a SCX bond elut and eluted with methanol (MeOH) followed by 10% 7M $NH_3$/MeOH in MeOH, followed by a reverse phase chromatography to give the titled compound (235 mg); $^1$H NMR (400MHz, $CDCl_3$) δ 1.07 (9H, s), 2.01 - 2.18 (2H, m), 2.22 (3H, s), 2.83 - 3.13 (4H, m), 3.35 - 3.89 (10H, m); LCMS *M*/z(+) 429 (*M*+$H^+$).

### Synthesis of phenyl *N*-(5-chloro-4-methyl-1,3-thiazol-2-yl)carbamate

To a suspension of 5-chloro-4-methyl-thiazol-2-amine hydrochloride (4.75 g) in $CH_2Cl_2$ under argon was added pyridine (4.16 mL). Phenylchloroformate (3.39 mL) was then added drop wise and the mixture was stirred for 3 hours. The reaction mixture was diluted with $CH_2Cl_2$ (200 mL) and 1M aqueous HCl (100 mL), stirred vigorously and then separated. The organic layer was washed with more 1M aqueous HCl (100 mL) then brine, dried ($Na_2SO_4$), filtered and concentrated to give a yellow solid which was heated to reflux temperature in 9:1 hexane-ethyl acetate (EtOAc) (100 mL) and filtered while hot to give the titled compound as a pale yellow solid (2.75g, 40%); LCMS *M*/z(+) 269 (*M*+$H^+$).

The following compounds were prepared according to the same method:

### 4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-(5-chlorothiazol-2-yl)piperazine-1-carboxamide (2a)

$^1$H NMR (400MHz, $CDCl_3$) δ 1.06 (s, 9H), 2.01 - 2.16 (m, 2H), 2.81 - 2.94 (m, 2H), 3.00 (d, 1H), 3.05 - 3.12 (m, 1H), 3.35 - 3.90 (m, 10H), 7.11 (s, 1H); LCMS *M*/z(+) 414.91 (*M*+$H^+$).

### *N*-(5-Chloro-4-isopropyl-thiazol-2-yl)-4-[(2*R*)-4-isopropylpiperazine-2-carbonyl]piperazine-1-carboxamide (2d)

$^1$H NMR (400MHz, $CDCl_3$) δ 1.0 (6H, m) 1.2 (6H, d) 2.1-2.25 (2H, m) 2.65-2.75 (2H, m) 2.8-2.9 (2H, m) 3.05-3.2 (2H, m) 3.4-3.8 (9H, m); LCMS *M*/z(+) 443 (*M*+$H^+$).

**4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-(5-chloro-4-cyclopropyl-thiazol-2-yl)piperazine-1-carboxamide (2e)**

$^1$H NMR (400MHz, d6-DMSO) δ 0.81 (2H, m), 0.93 (2H, m), 1.01 (9H, s), 1.90-2.20 (4H, m), 2.70 (2H, m), 2.83 (1H, m), 2.98 (2H, m), 3.40-3.90 (7H, m); LCMS *M*/z(+) 455 (*M*+H$^+$).

**4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-[5-chloro-4-(trifluoromethyl)-1,3-thiazol-2-yl]piperazine-1-carboxamide (2f)**

$^1$H NMR (400MHz, $CDCl_3$) δ ppm 1.06 (9H, s), 2.02 - 2.15 (2H, m), 2.82 - 2.94 (2H, m), 3.00 (1H, d), 3.06 - 3.13 (1H, m), 3.37 - 3.95 (10H, m). LCMS *M*/z(+) 483 (*M*+H$^+$).

**4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-(4-cyclopropylthiazol-2-yl)piperazine-1-carboxamide (2h)**

$^1$H NMR (400MHz, d6-DMSO) δ 0.73 (2H, m), 0.80 (2H, m), 0.99 (9H, s), 1.85-2.07 (4H, m), 2.63 (2H, m), 2.69 (1H, m), 2.91 (2H, m), 3.40-3.70 (7H, m), 6.59 (1H, s); LCMS *M*/z(+) 421 (*M*+H$^+$).

***N*-(4-tert-Butyl-5-fluoro-thiazol-2-yl)-4-[(2*R*)-4-isopropylpiperazine-2-carbonyl]piperazine-1-carboxamide (2i)**

$^1$H NMR (400MHz, $CDCl_3$) δ 1.1 (6H, m), 1.3 (9H, s), 2.2-2.4 (2H, m), 2.8-3.0 (4H, m), 3.1 (1H, m), 3.5-4.0 (9H, m); LCMS *M*/z(+) 441 (*M*+H$^+$).

**4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-(5-methoxy-4-methyl-thiazol-2-yl)piperazine-1-carboxamide (2k)**

$^1$H NMR (400MHz, $CDCl_3$) δ 1.0 (9H, s) 2.0-2.05 (2H) 2.1 (3H, s) 2.8-2.9 (2H, m) 2.95 (1H, m) 3.05 (1H, m) 3.4-3.75 (9H, m) 3.8 (3H, s). LCMS *M*/z(+) 425 (*M*+H$^+$).

***N*-(5-Bromo-4-methyl-1,3-thiazol-2-yl)-4-[(2*R*)-4-tert-butylpiperazine-2-carbonyl]piperazine-1-carboxamide (2l)**

$^1$H NMR (400MHz, $CDCl_3$) δ ppm 1.0 (9H, m), 2.0-2.1 (2H, m), 2.15 (3H, s), 2.8-2.85 (2H, m), 2.9-2.05 (2H, m), 3.3-3.8 (9H, m). LCMS *M*/z(+) 475 (*M*+H$^+$).

**Synthesis of *N*-(5-chloro-4-ethyl-thiazol-2-yl)-4-[(2*R*)-4-isopropylpiperazine-2-carbonyl]piperazine-1-carboxamide (2c)**

*N*-(5-Chloro-4-ethyl-1,3-thiazol-2-yl)piperazine-1-carboxamide (179 mg) was added to a stirred solution of (2*R*)-1-tert-butoxycarbonyl-4-isopropyl-piperazine-2-carboxylic acid (177 mg) in THF (10 mL). 1- Hydroxybenzotriazole (100 mg), 1 -(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (125 mg) and *N*,*N*-diisopropylethylamine (114 µL) were then added and the mixture was left to stir at room temperature overnight. The reaction mixture was evaporated to dryness then re-dissolved in $CH_2Cl_2$, washed with 2M aqueous NaOH, then brine, dried ($MgSO_4$) and concentrated under reduced pressure to give 500 mg of material. This was purified on a 20 g silica bond elut eluting with 50% EtOAc in hexane to EtOAc + 20% MeOH to give a white solid. The solid was re-dissolved in $CH_2Cl_2$, TFA was added and the mixture was stirred at room temperature for 2h before being transferred into a SC bond elut eluting with MeOH then 10% $NH_3$ in MeOH to give the titled

compound (60 mg, 19%) as a pale yellow oil. $^1$H NMR (400MHz, $CDCl_3$) δ 1.05 (6H, dd) 1.15-1.25 (3H, m) 2.1-2.3 (2H, m) 2.55-2.65 (2H, m) 2.7-2.8 (2H, m) 2.85-3.0 (2H, m) 3.05-3.15 (1H, m) 3.5-3.8 (9H, m) 3.85-3.90 (1H, m). LCMS *M*/z(+) 429 ($M$+$H^+$).

**Synthesis of *N*-(5-chloro-4-ethyl-1,3-thiazol-2-yl)piperazine-1-carboxamide**

To a solution of tert-butyl piperazine-1-carboxylate (198 mg) in $CH_2Cl_2$ (10 mL) was added triethylamine (444 µL) followed by phenyl *N*-(5-chloro-4-ethyl-1,3-thiazol-2-yl)carbamate (299 mg) and the resulting mixture was heated at 60°C for 1 hour. The mixture was washed with water and the organics were dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by silica chromatography eluting with a gradient of ethyl acetate/isohexane (0 to 50%). The resulting product was dissolved in $CH_2Cl_2$ (5 mL), TFA (2 mL) was added and the resulting mixture was stirred at room temperature for 2 hours. The reaction mixture was transferred to a SCX column and eluted with methanol, followed by 10% 7M $NH_3$ in methanol to afford the titled compound (90 mg). $^1$H NMR (400.132 MHz, $CDCl_3$) δ ppm 1.2 (3H, t), 2.6 (2H, q), 2.9 (4H, m), 3.5 (4H, m). LCMS *M*/z(+) 275 ($M$+$H^+$).

The following compounds were prepared according to the same method:

***N*-(4-tert-Butyl-5-chloro-thiazol-2-yl)-4-[(2*R*)-4-isopropylpiperazine-2-carbonyl]piperazine-1-carboxamide (2g)**

$^1$H NMR (400MHz, $CDCl_3$) δ 1.0 (4H, dd) 1.4 (9H, s) 2.1-2.25 (2H, m) 2.65-2.95 (4H, m) 3.05-3.10 (1H, m) 3.4-3.8 (9H, m). LCMS *M*/z(+) 457 ($M$+$H^+$).

**4-[(2*R*)-4-tert-Butylpiperazine-2-carbonyl]-*N*-(5-fluorothiazol-2-yl)piperazine-1-carboxamide (2j)**

$^1$H NMR (400MHz, $CDCl_3$) δ 1.08 (s, 9H), 2.04 - 2.20 (m, 2H), 2.86 - 2.97 (m, 2H), 3.00 - 3.15 (m, 2H), 3.40 - 3.89 (m, 9H), 6.89 (d, 1H). LCMS *M*/z(+) 399 ($M$+$H^+$).

## Assays

**CCR2 receptor inhibition assay**

A 1 mL aliquot of THP-1 cells stably expressing human CCR2 in RPMI 1640 cell culture medium (0.85 x $10^7$ cells/mL) was diluted to 50 mL with HPSS buffer containing 0.1% BSA and the tube spun at 1200 rpm for 5 minutes. The supernatant was aspirated off and the residue resuspended in 13 mL 0.1% BSA buffer containing 25 µL pluronic acid (20% in DMSO) and 25 µLFluo-4 dye (1 mg/442 µL) and incubated at 37°C for 40 mins in a humidified chamber (5% $CO_2$/95% air). 30 µL of the 0.65 x $10^6$ cells/mL cell suspension was pipetted into all wells of a 384 CellBIND (Corning 3683) plate containing 10 µL test compound solution (prepared by serial dilution from 10 mM stock on dimethyl sulfoxide) in buffer and the plate incubated at 37°C for 10 mins in 5% $CO_2$.The plate was centrifuged at 1200 rpm for 5 mins the incubated at 37°C for 10-15 mins in 5% $CO_2$. CCL2 was added in buffer at

a concentration equal to its $EC_{50}$ value and mobilization of intracellular calcium was measured online (FLIPR, Molecular Devices). $IC_{50}$ values were determined using in-house software.

**Glutathione reactivity assay**

Stock solutions (1.5 mM in a 50:50 mixture of acetonitrile and 2-methoxyethanol) of the compounds to be tested were prepared from solid samples supplied from the AstraZeneca compound collection. All other chemicals were used as supplied from Sigma Aldrich. To measure the reactivity of the compounds towards glutathione, the compound stock solution was added to pH 7.4 phosphate buffer in 2 mL amber HPLC vials at 37°C containing 5 mM glutathione and 1 mM ethylenediaminetetraacetic acid, to give a final concentration of 50 μM (final volume of 1.5 mL). The rate of parent loss was then measured by LC-UV (Waters Alliance HT LC pump with Thermo Spectra system UV 6000LP 5cm diode array using Chromquest software v 4.1) over a period of 18 hours. All liquid handling steps were performed by a Gilson 232XL liquid handler using 735 Sample Software v3.10. Where parent loss was observed, the peak area data was then fitted assuming pseudo-first order kinetics in Microsoft Excel to give a half-life for the reaction under these conditions. Confirmation of the identity of the parent and, where possible, the products of the reactions, was performed using a Water Micromass ZQ mass spectrometer. To test for any hydrolysis of the compounds under these conditions, the above procedure was repeated for all compounds in the absence of glutathione. To check the viability of the glutathione, p-nitrobenzyl chloride was used as a positive control in each experiment.