Supplementary material

Synthesis of 6-(piperidin-1-ylcarbonyl)quinoxaline (CX516)

CX516 was synthesized according to Burdeniuc (2003) and verified by ^{1}H and ^{13}C NMR and elemental analysis: ^{1}H NMR (400 MHz, DMSO): 9.01 (2H, s), 8.16 (1H, d, J=8.5 Hz), 8.05 (1H, d, J=1.9 Hz), 7.84 (1H, dd, J=1.9, 8.5 Hz), 3.65 (2H, s), 3.30 (2H, s), 1.61 (4H, s), 1.47 (2H, s). ^{13}C NMR (400 MHz, DMSO): 167.5, 146.5, 146.5, 142.1, 141.7, 138.0, 129.6, 128.5, 126.8, 48.0, 42.3, 25.9, 24.2, 24.0. Elemental analysis: Anal. calcd for $C_{14}H_{15}N_3O$: C 69.69, H 6.27, N 17.41; found: C 69.71, H 6.33, N 17.79.

Synthesis of (±)-(3-methyl-1-piperidyl)-quinoxalin-6-yl-methanone (Me-CX516)

(±)-3-Methylpiperidine (1.51 g, 15.1 mmol) in THF (5 ml) was added dropwise to a mixture of quinoxaline-6-carbonyl chloride (3.0 g, 13.1 mmol), triethylamine (3.2 g, 31.4 mmol) and THF (50 ml) at room temperature followed by stirring for 1 h at room temperature. Water (100 ml) was added followed by extraction twice with ethyl acetate (2 X 100 ml). The organic phase was washed with aqueous sodium hydroxide (50 ml, 0.5 *M*), saturated aqueous sodium chloride (50 ml) and water (50 ml). The mixture was dried with magnesium sulfate and evaporated to give Me-CX516 as yellow oil (2.0 g, 60%). Me-CX516 hydrochloride was precipitated by adding concentrated hydrochloric acid (3 ml, 3 *M*) dissolved in ethanol to a stirred mixture of the free base above and diethylether (10 ml). Me-CX516 hydrochloride was obtained as a yellow solid (0.73 g, 19%). Mp 95-100 °C. The final compound was verified by ^{1}H and ^{13}C NMR as well as LC-ESI-HRMS and elemental analysis: ^{1}H NMR (400 MHz, DMSO): 9.01 (2H, s), 8.17 (1H, d, J=8.6 Hz), 8.05 (1H, d, J=1.8 Hz), 7.83 (1H, dd, J=1.8, 8.6 Hz), 4.37 - 4.36 (1H, m), 3.56 - 3.41 (1H, m), 3.11 - 3.01 (0.5H, m), 2.95 - 2.86 (0.5H, m), 2.80 - 2.71 (0.5H, m), 2.62 - 2.52 (0.5H, m), 1.81 - 1.77 (1H, m), 1.74 - 1.37 (3H, m), 1.23 - 1.11 (1H, m), 0.95-0.94 (1.5H, m) 0.71-0.68 (1.5H, m). ^{13}C NMR (400 MHz,

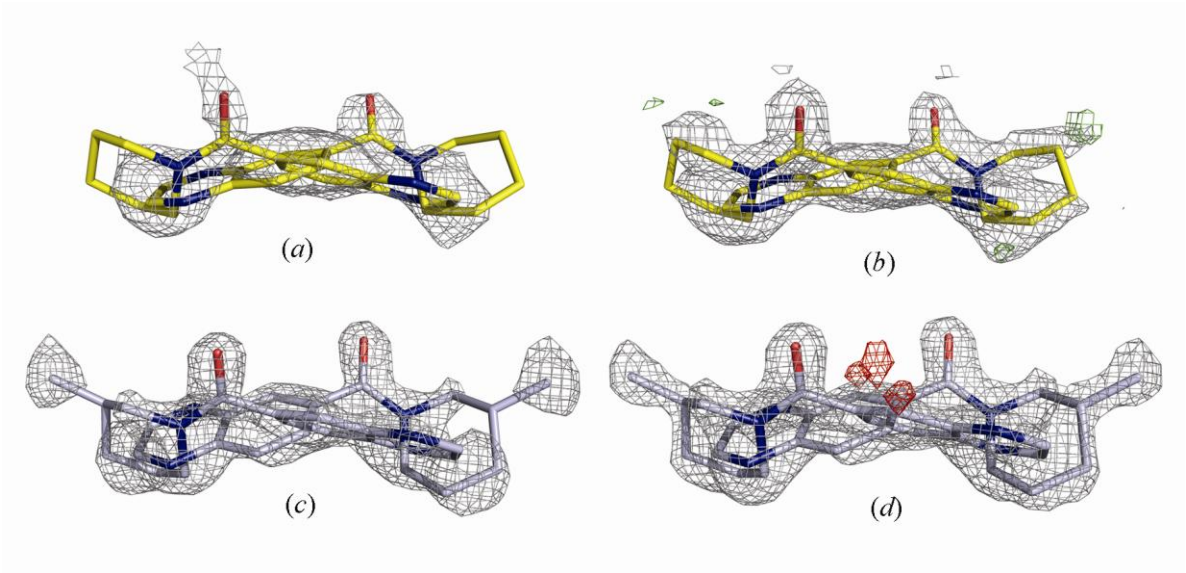
DMSO): 167.5, 146.5, 146.5, 142.1, 141.7, 138.0, 129.6, 128.6, 126.8, 54.9, 54.1, 48.6, 47.5, 41.9, 32.4, 31.1, 30.5, 25.2, 24.2, 18.9, 18.4. LC-ESI-HRMS m/z $[M^{+}H]^{+}$ calcd for $C_{15}H_{17}N_3O$: 256.1450; found 256.1443. Elemental analysis: Anal. calcd for $C_{15}H_{17}N_3O \cdot 3/4H_2O$: C 67.02, H 6.94, N 15.43; found: C 67.03, H 6.43, N 15.47.

Instrumentation

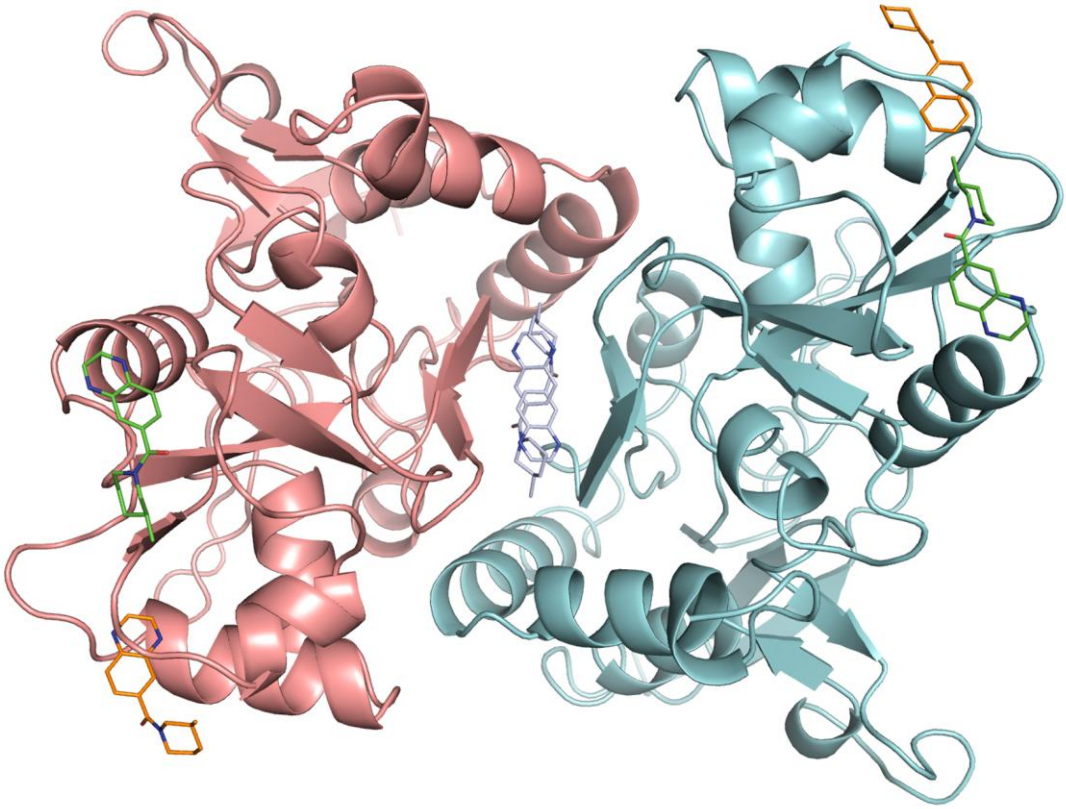
Melting points were obtained on a Mettler FP81HT MCB Cell connected to Mettler Toledo FP90 Central Processor. All NMR spectra were recorded on a 400 MHz Bruker Avance II instrument. Chemical shifts values are expressed in ppm, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Coupling constants (J) are reported in hertz (Hz). LC-ESI-HRMS data were recorded on a Shimadzu UFLC prominence coupled to a Bruker Daltonics MaXis Q-TOF.

Compound purity

The purity of CX516 and Me-CX516 was determined by elemental analysis to a deviation from calculated values within 0.4%, except for H content in Me-CX516 that deviates 7.9%.



Supplementary Figure S1. Electron-density maps for CX516 (panels *a* and *b*) and (*R*)-Me-CX516 (panels *c* and *d*). Panels (*a*) and (*c*) show 2mFo−DFc electron-density maps (grey; contoured at 1σ level; 0.41 e Å^{-3} for CX516 and 0.41 e Å^{-3} for (*R*)-Me-CX516) resulting from the ARP/wARP autobuild before introduction of modulators. Panels (*b*) and (*d*) show the final 2mFo−DFc electron-density maps (grey; contoured at 1σ level; 0.40 e Å^{-3} for CX516 and 0.41 e Å^{-3} for (*R*)-Me-CX516) and the final mFo−DFc maps (green for positive density and red for negative density, contoured at 3σ level; ± 0.35 e Å^{-3} for CX516 and ± 0.33 e Å^{-3} for (*R*)-Me-CX516). Final coordinates for CX516 and (*R*)-Me-CX516 are shown in yellow and light blue stick representation, respectively.



Supplementary Figure S2. Additional binding sites for Me-CX516. GluA2 LBD-L483Y-N754S is shown in cyan cartoon representation along with its symmetry related molecule shown in salmon cartoon representation. Me-CX516 bound at the dimer interface is shown in light blue stick representation. The additional Me-CX516 molecules bound in the vicinity of Lys117 and Lys185 and in the vicinity of Thr131 and Tyr161 are shown in green and orange stick representation, respectively. These are modeled as low energy conformations of the (*S*)-enantiomer. However, density for the piperidine rings is not well defined at 1σ (0.41 e $Å^{-3}$).

Reference

Burdeniuc, J.J. (2003): Method for preparing heterocyclic-carboxylic acids. European patent application EP 1 277 739 A1. European patent office 2003.