SUPPLEMENTARY DATA

Retinoic-acid-orphan-receptor-C inhibition suppresses Th17 cells and induces thymic aberrations

Synthesis of cpds 1 and 2:

All reagents and solvents were purchased from commercial suppliers and used without further purification. All reactions were performed under inert conditions (nitrogen) unless otherwise stated. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 400 MHz or a Bruker 600 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the internal solvent reference. Significant peaks are tabulated in the order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quintet; m, multiplet; br, broad), coupling constants, and number of protons. Final compounds were purified to ≥ 95% purity as assessed by analytical liquid chromatography with the following method: Waters Acquity UPLC−MS; column HSS T3 1.8 μm, 2.1 mm × 50 mm; A, water + 0.05% formic acid + 3.75 mM ammonium acetate; B, acetonitrile + 0.04% formic acid; 5−98% B in 1.4 min, 98% B 0.45 min, flow 1.0 ml/min; column temperature 60°C.

Synthesis of cpd 1, *(S)-N-(5-chloro-3-((4-(cyclopentanecarbonyl)-3-methylpiperazin-1-yl)methyl)-2-methylphenyl)-2-methylpyrimidine-5-carboxamide*, was done according to the procedures described in the patent application WO 2014086894 (example 192). LCMS R_t 1.02 min; MS m/z 469.0

$(M+H)^+$. 1H NMR (400 MHz, DMSO-d_6, 100 °C) δ 10.00 (s, 1H), 9.18 (s, 2H), 7.45 (d, J = 2.1 Hz, 1H), 7.30 (d, J = 2.0 Hz, 1H), 4.44 (br s, 1H), 3.99 (br s, 1H), 3.51 (s, 2H), 3.01 – 2.91 (m, 1H), 2.83 – 2.75 (m, 1H), 2.74 (s, 3H), 2.73 – 2.64 (m, 1H), 2.28 (s, 3H), 2.26 – 2.14 (m, 1H), 2.07 – 1.97 (m, 1H), 1.82 – 1.72 (m, 4H), 1.68 – 1.58 (m, 4H), 1.62 – 1.52 (m, 2H), 1.22 (d, J = 6.6 Hz, 2H).

Synthesis of cpd 2, *1-(2,6-dichlorophenyl)-2-(furan-2-yl)-4-isobutyl-5-methyl-1H-imidazole* (Supplementary Figure 1). *2-Bromo-5-methylhexan-3-one* (**3**): A solution of 5-methylhexan-3-one (5 g, 43.8 mmol) in methanol (30 ml) was cooled to – 15 °C. Br_2 (2.26 ml, 43.8 mmol) was added and the solution stirred for 30 minutes. Then, the reaction mixture was warmed to 15°C and after another 30 minutes to room temperature (rt). The color of the solution started to turn from red to yellow. The reaction mixture was stirred at rt for another 1.5 hours and then poured onto sat. $NaHCO_3$-soln. (50 ml) and extracted with EtOAc/cHex 3:1 (3 x 50 ml). The combined organic phases were washed with 10% Na_2SO_3-soln. (50 ml) and water (50 ml) and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel and eluted with 0-50% of DCM/cHex to obtain 7.3 g (57%) of a 7:3 mixture of **3** and the corresponding regioisomer **4**. The mixture of regioisomers was used for the next step without further purification. Regioisomer **3**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.76 (q, J = 6.7 Hz, 1H), 2.65 – 2.54 (m, 2H), 2.06 (dq, J = 13.4, 6.7 Hz, 1H), 1.63 (d, J = 6.7 Hz, 3H), 0.88 (app. t, J = 7.3 Hz, 6H). Regioisomer **4**: 4.57 (d, J = 7.4 Hz, 1H), 2.75 – 2.63 (m, 2H), 2.19 (dq, J = 13.3, 6.6 Hz, 1H), 1.01 – 0.96 (m, 6H), 0.93 (d, J = 6.6 Hz, 3H).

N-(2,6-dichlorophenyl)furan-2-carboximidamide (**5**): To a stirred yellow solution of NaHMDS (1M in THF, 95 ml, 95 mmol) was added dropwise under argon atmosphere a solution of 2,6-dichloroaniline (15 g, 91 mmol) in THF (25 ml) over 10 minutes and stirring continued for 1.5 hours at rt. Then, a solution of 2-furonitrile (8.96 g, 95 mmol) in THF (37 ml) was added dropwise over 20 minutes. The solution turned dark during addition and the resulting black mixture was stirred for another 30 minutes at rt. The solvent was removed under reduced pressure yielding a brown solid which was filtered by column chromatography on silica gel using 1-20% of EtOAc/cHex as eluent to yield **5** (19.3 g, 82%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.81 (s, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.25 – 7.03 (br s, 1H), 6.98 (t, J = 8.0 Hz, 1H), 6.61 (br m, 3H).

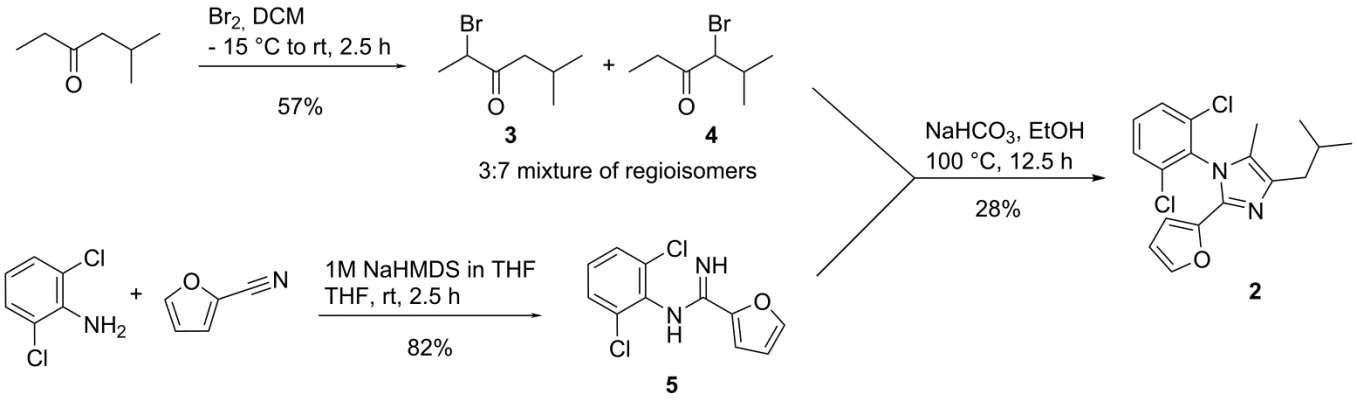
1-(2,6-dichlorophenyl)-2-(furan-2-yl)-4-isobutyl-5-methyl-1H-imidazole (**2**): In a microwave vial, carboxamideimide **5** (850 mg, 3.3 mmol) and bromide **3** (1.65 g, 6.0 mmol, containing 30% of regioisomer **4**) were dissolved in EtOH (15 ml) and $NaHCO_3$ (980 mg, 11.7 mmol) was added. The vial was sealed and the solution heated to 100 °C for 7.5 hours. Another portion of bromide **3** (919 mg, 3.3 mmol, containing 30% of regioisomer **4**) and $NaHCO_3$ (280 mg, 3.3 mmol) were added, the vial recapped and heated to 100 °C for another 5 hours. The reaction mixture was quenched with sat. $NaHCO_3$-soln. and extracted with EtOAc (2 times), the combined organics washed with brine, dried (Na_2SO_4) and concentrated under vacuum. The yellow residue was purified by column chromatography on silica gel and eluted with 0-30% of acetone/cHex to obtain **2** as a white solid (333 mg, 28%). LCMS R_t 1.25 min; MS m/z 349.2 $[M+H]^+$. ^{1}H NMR (400 MHz,

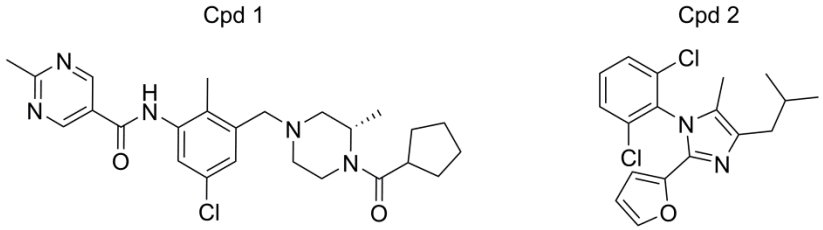
Methanol-d_4) δ 7.68 (d, J = 9.1 Hz, 1H), 7.67 (d, J = 7.0 Hz, 1H), 7.60 (dd, J = 9.1, 7.0 Hz, 1H), 7.40 (dd, J = 1.8, 0.7 Hz, 1H), 6.40 (dd, J = 3.5, 1.8 Hz, 1H), 6.16 (dd, J = 3.5, 0.7 Hz, 1H), 2.50 (d, J = 7.3 Hz, 2H), 2.06 (s, 1H), 1.96 (s, 3H), 0.99 (d, J = 6.7 Hz, 6H). 13C NMR (151 MHz, DMSO-d_6) δ 144.82, 142.95, 137.57, 135.98, 133.90, 132.30, 132.15, 129.28, 124.22, 111.40, 106.86, 35.69, 28.71, 22.10, 8.10.

Supplementary Figure 1: Chemical structures of cpd 1 and cpd 2 as well as reagents and conditions for synthesis of cpd 2. Abbreviations: DCM, dichloromethane; EtOH, ethanol; NaHMDS, sodium hexamethyldisilazane; THF, tetrahydrofuran.

Supplementary Figure 2: Representative T cell receptor spectratypes of Vβ8.1/8.2 showing an intense oligoclonal band for Nb11.2 (right panel) whereas polyclonal signal is observed for the cpd 1-treated rat showing cortical hyperplasia (left panel). No differences were observed for all other Vβ spectratypes (not shown). Blue = FAM-labeled PCR products, orange = size standard.

Supplementary Figure 1





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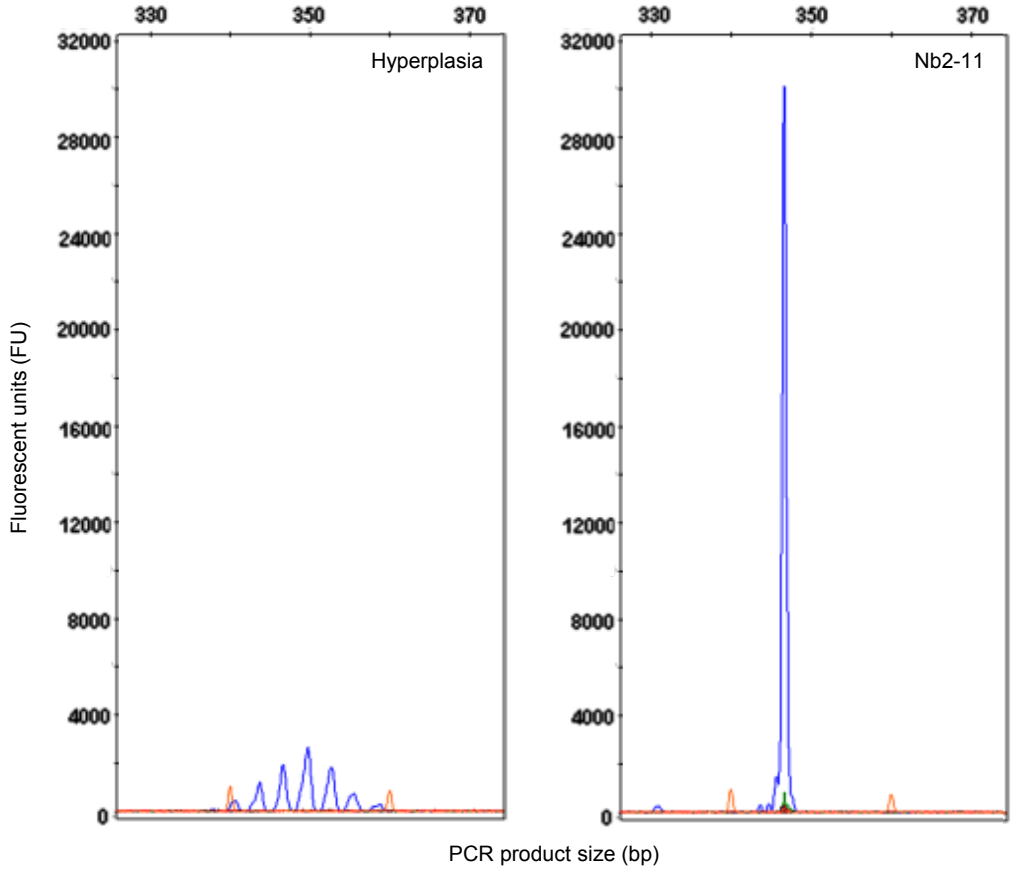
Supplementary Figure 2



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