Supporting Information

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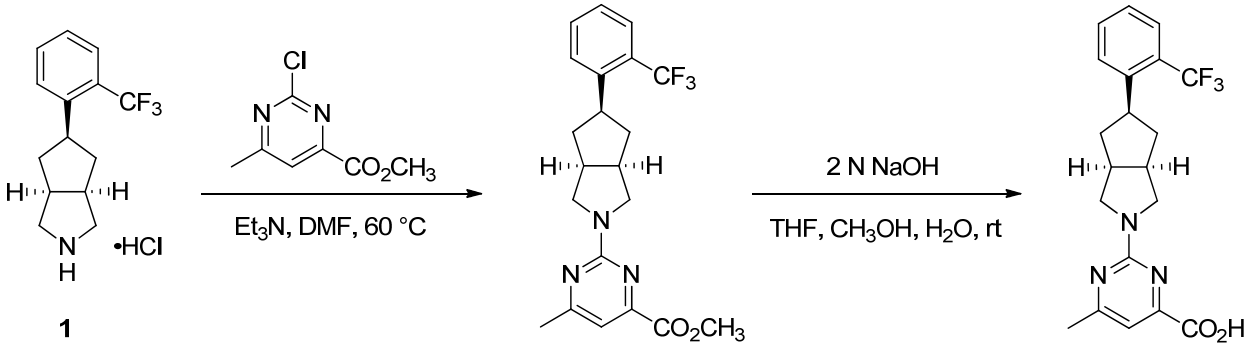
General Chemistry Information

All reactions were performed under a dry atmosphere of nitrogen unless otherwise specified. Indicated reaction temperatures refer to the reaction bath, while room temperature is noted as 25 °C. Commercial grade reagents and anhydrous solvents were used as received from vendors and no attempts were made to purify or dry these components further. Removal of solvents under reduced pressure was accomplished with a Buchi rotary evaporator at approximately 28 mm Hg pressure using a Teflon-linked KNF vacuum pump. Thin layer chromatography was performed using 1" x 3" AnalTech No. 02521 silica gel plates with fluorescent indicator. Visualization of TLC plates was made by observation with either short wave UV light (254 nm lamp), 10% phosphomolybdic acid in ethanol or in iodine vapors. Flash column chromatography was carried out using a Teledyne Isco CombiFlash Companion Unit with RediSep®Rf silica gel columns. Proton NMR spectra were obtained either on 300 MHz Bruker Nuclear Magnetic Resonance Spectrometer and chemical shifts (δ are reported in parts per million (ppm) with the following spectral pattern designations: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet; br, broad. Tetramethylsilane was used as an internal reference. Melting points are uncorrected and were obtained using a MEL-TEMP Electrothermal melting point apparatus. Mass spectroscopic analyses were performed using positive mode electron spray ionization (ESI) on a Varian ProStar LC-MS with a 1200L quadrupole mass spectrometer. High pressure liquid chromatography (HPLC) purity analysis was performed using a Varian Pro Star HPLC system with the following parameters (Method A): a binary solvent system A and B using a gradient elusion [A, H_2O with 0.025% trifluoroacetic acid (TFA); B, CH_3CN with 0.025% TFA]; flow rate = 1 mL/min; elution time of 20 minutes; UV detection at 254 nm using a Phenomenex C18(2) column (250 × 4.6 mm). Combustion analysis was conducted by Intertek Pharmaceutical Services, P.O. Box 470, Salem Industrial Park Bldg. #5, Whitehouse, NJ 08888.

Synthesis of BPN-14136

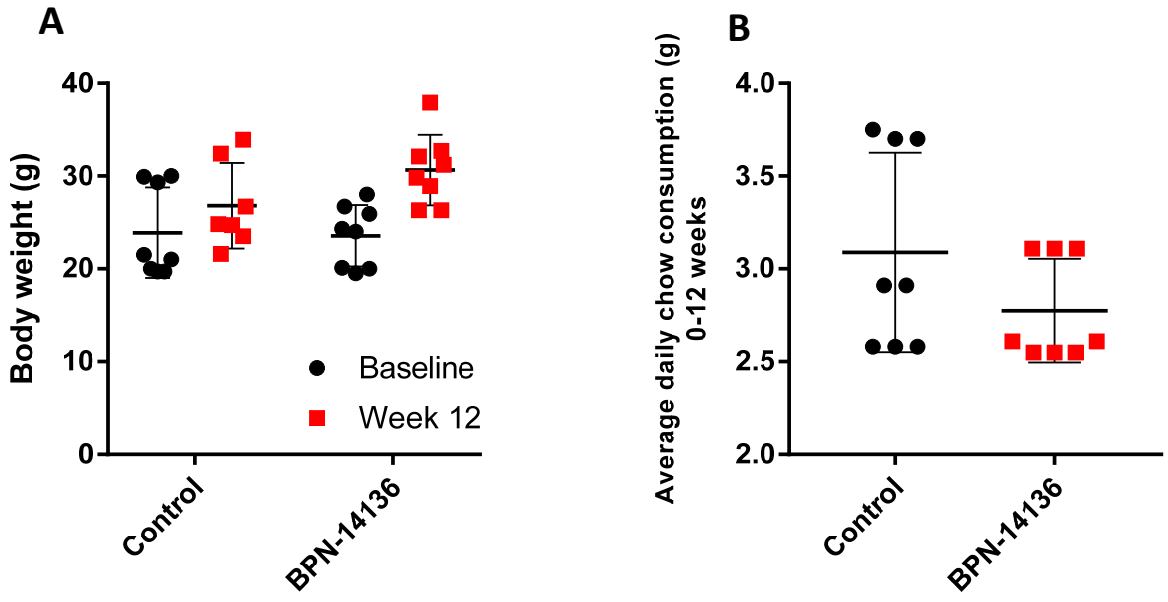


1 **2** **3**

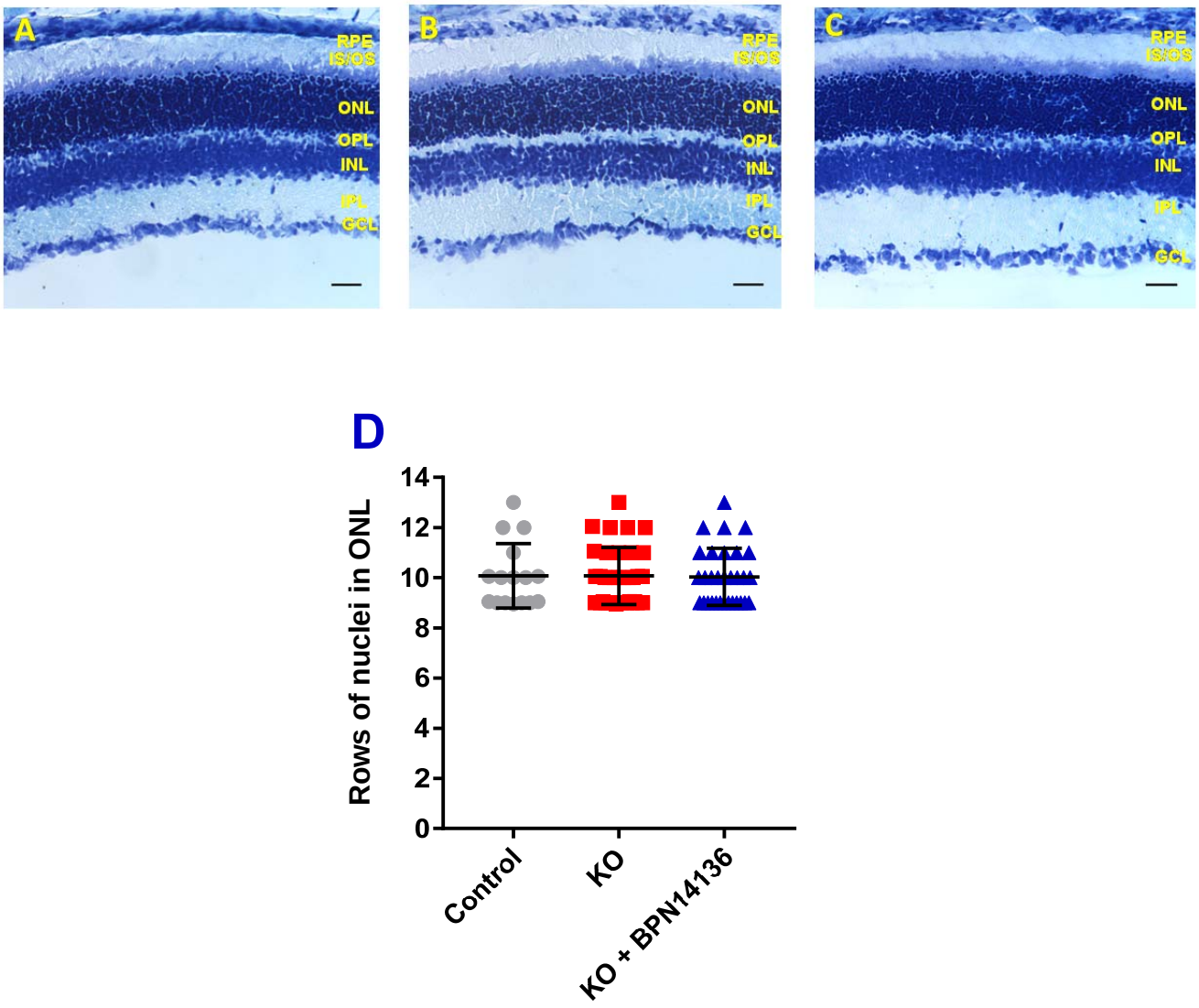
*(3aR,5R,6aS)-5-(2-(Trifluoromethyl)phenyl)octahydrocyclopenta[c]pyrrole Hydrochloride (**1**):* Compound **1** was synthesized in eight steps from (3a*R*,7a*S*)-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione as described for compound **26** in our previous publication (1)

*6-Methyl-2-((3aR,5r,6aS)-5-(2-(trifluoromethyl)phenyl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)pyrimidine-4-carboxylic Acid (**3**):* Step A: To a solution of (3a*R*,5*R*,6a*S*)-5-(2-(trifluoromethyl)phenyl)octahydrocyclopenta[c]pyrrole hydrochloride (1) (**1**, 1.0 g, 3.43 mmol) and Et_3N (1.43 mL, 10.29 mmol) in DMF (50 mL) was added methyl 2-chloropyrimidine-4-carboxylate (0.641 g, 3.43 mmol), and the resulting mixture stirred at 60 °C for 16 h. The mixture was allowed to cool to room temperature and was diluted with H_2O (200 mL). The resulting aqueous mixture was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed with H_2O (3 × 100 mL), brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was chromatographed over silica gel (0% to 30% EtOAc in hexanes) to give methyl 6-methyl-2-((3a*R*,5*R*,6a*S*)-5-(2-(trifluoromethyl)phenyl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)pyrimidine-4-carboxylate (**2**) as an off-white solid (1.20 g, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (m, 1H), 7.58 (m, 2H), 7.23 (m, 1H), 7.05 (s, 1H), 3.95 (s, 3H), 3.82 (m, 4H), 3.59 (m, 1H), 2.92 (m, 2H), 2.44 (s, 3H), 2.40 (m, 2H), 1.69 (m, 2H); MS (ESI+) *m/z* 406 $[M + H]^+$.

Step B: To a solution of 6-methyl-2-((3a*R*,5*R*,6a*S*)-5-(2-(trifluoromethyl)phenyl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)pyrimidine-4-carboxylate (**2**, 1.2 g, 2.95 mmol) in a 1:1 mixture of CH_3OH:THF (40 mL) was added aqueous 2 N NaOH solution (20 mL), and the resulting mixture stirred at room temperature for 16 h. The mixture was carefully neutralized at 0 °C to pH = 7 via slow addition of an aqueous 2 N HCl solution. The resulting aqueous mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the combined organic extracts were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was chromatographed over silica gel (0% to 10% CH_3OH in CH_2Cl_2) to give 6-methyl-2-((3aR,5r,6aS)-5-(2-(trifluoromethyl)phenyl)hexahydrocyclopenta[c]pyrrol-2(1H)-yl)pyrimidine-4-carboxylic acid (**3**) as a light-yellow solid (1.0 g, 86%): melting point = 129–131 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.30 (bs, 1H), 7.71–7.58 (m, 3H), 7.40 (m, 1H), 7.01 (s, 1H), 3.73–3.61 (m, 4H), 3.35 (m, 1H), 2.87 (m, 2H), 2.37 (s, 3H), 2.24 (m, 2H), 1.66 (m, 2H); MS (ESI+) *m/z* 392 $[M + H]^+$; HPLC >99% purity (AUC), t_R = 18.49 min (Method A); combustion analysis (%CHN): calculated for $C_{20}H_{20}F_3N_3O_2$•0.25H_2O: %C = 60.68; %H = 5.22; %N = 10.46; found: %C = 60.66; %H = 4.95; %N = 10.46.



Supplementary Figure S1. Chronic BPN-14136 administration in *Abca4*$^{-/-}$ mice does not reduce body weight and chow consumption. (*A*), Body weight at baseline and at the end of the 12-week dosing period in vehicle-treated *Abca4*$^{-/-}$ mice (control) in comparison to the BPN-14136-treated *Abca4*$^{-/-}$animals (BPN-14136). (*B*), Average daily chow consumption over the 12-week treatment period in vehicle-treated *Abca4*$^{-/-}$ mice (control) and in the BPN-14136-treated *Abca4*$^{-/-}$animals (BPN-14136). At week 12, the mean body weight was not significantly different in the compound-treated and control groups (unpaired parametric t-test, P=0.0997). Chow consumption was not significantly different in the treated and control groups (unpaired parametric t-test, P=0.165). No weight loss or statistically significant reduction in chow consumption in the compound-treated animals in comparison to the control group is consistent with the lack of systemic toxicity for BPN-14136.



Supplementary Figure S2. Analysis of RPE and retina cryosections by light microsopy. Toluidine blue staining of the representative retinas sections from wild type mice (*A*) and vehicle-treated *Abca4*$^{-/-}$ mice (*B*) in comparison to the BPN-14136-treated *Abca4*$^{-/-}$animals (*C*). No abberant morphology was revealed in the retina after 12 weeks of BPN-14136 administration at the 20 mg/kg dose. Scale bar: 25 µm. (*D*), Quantification of nuclei spanning the width of the outer nuclear layer (ONL). The number of photoreceptor nuclei in the ONL was counted in central mouse retina from wild type aniamls (3 eyes; 16 sections), vehicle-treated *Abca4*$^{-/-}$ mice (3 eyes; 17 sections), and BPN-14136-treated *Abca4*$^{-/-}$animals (3 eyes; 17 sections). Consistent with the retinal safety of BPN-14136, no statistically significant difference between the three treatment groups was found (one-way ANOVA with Holm-Sidak *post-hoc* test analysis, P=0.9997, alpha = 0.05).

Supplementary Table S1. BPN-14136 plasma levels following a single oral 5 mg/kg dose administration

Animal ID	Time (hr)	Plasma Conc., ng/ml		SD
		Individual	Mean	
16	0.083	6020		
17	0.083	6880		
18	0.083	2900	5267	2094
19	0.25	10180		
20	0.25	8000		
21	0.25	13320	10500	2674
22	0.5	11360		
23	0.5	9860		
24	0.5	8440	9887	1460
16	1	7360		
17	1	9160		
18	1	6800	7773	1233
25	2	11960		
26	2	9400		
27	2	11720	11027	1414
19	4	11700		
20	4	7320		
21	4	7460	8827	2489
22	8	7540		
23	8	6320		
24	8	4720	6193	1414
25	12	4300		
26	12	6980		
27	12	4700	5327	1446
28	24	340		
29	24	116		
30	24	576	344	230
28	48	ND[a]		
29	48	23.4		
30	48	ND[a]	23.4	NA[b]

Dosing cohorts consisted of three groups of drug naïve adult male CD-1 mice. Plasma Lower Limit of Quantitation (LLOQ) = 10.0 ng/ml. [a] ND: No data; [b] NA: Not applicable

Supplemental Reference

1. Cioffi CL, R. B., Freeman EE, Conlon MP, Chen P, Stafford DG, Schwarz DM, Zhu L, Kitchen DB, Barnes KD, Dobri N, Michelotti E, Cywin CL, Martin WH, Pearson PG, Johnson G, Petrukhin K. . (2015) Bicyclic [3.3.0]-Octahydrocyclopenta[c]pyrrolo Antagonists of Retinol Binding Protein 4: Potential Treatment of Atrophic Age-Related Macular Degeneration and Stargardt Disease. *J Med Chem* **58**, 5863−5888