Molecular Pharmacology

A selective high affinity antagonist of the $P2Y_{14}$ receptor inhibits UDP-glucose-stimulated chemotaxis of human neutrophils

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Supplemental Experimental Procedures

General Methods. All solvents (regular and anhydrous) were of analytical grade, obtained from commercial suppliers and used without further purification. All other reagents were of analytical grade, and were purchased from Sigma-Aldrich (St. Louis, MO). Compound **1** (ethyl-7-bromo-4-hydroxy-2-naphthoate) was synthesized as described by Boger and co-workers,[1] and 4-(4-bromophenyl)3,6-dihydro 2H-pyridine-1-carboxylic acid *tert*-butyl ester was synthesized from the commercially available 4-(4-bromophenyl)-4-piperidinol. This synthetic route was according to Belly et al.[2] Reactions were conducted under an atmosphere of nitrogen whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with a fluorescent indicator, which were visualized: (a) under UV light or (b) by dipping in a $KMnO_4$ solution (40 g of K_2CO_3 and 6g of $KMnO_4$ in 600mL of water, then 5mL of 10% NaOH added). Column chromatography was performed on silica gel (SiO_2, 200-400 mesh, 60 Å) using moderate air pressure. After column chromatography, the appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 12 h to give the desired products in high purity. ^{1}H NMR spectra were recorded with a

Bruker Avance 400 MHz NMR spectrometer using D_2O, $CDCl_3$ or MeOH-d_4 as a solvent. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or using deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26 ppm). ESI-high resolution mass spectroscopic (HRMS) measurements were performed on a proteomics-optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine. Observed mass accuracies are those expected on the basis of the known performance of the instrument as well as the trends in masses of standard compounds observed at intervals during the series of measurements. Reported masses are observed masses uncorrected for this time-dependent drift in mass accuracy. Purity of compounds was checked using a Hewlett–Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μm analytical column (50 x 4.6 mm; Agilent Technologies Inc, Palo Alto, CA). Mobile phase: linear gradient solvent system: 5 mM TBAP (tetrabutylammonium dihydrogen phosphate)-CH_3CN from 80:20 to 40:60 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector at 254, 275, and 280 nm. Compound **6** (PPTN) was purified HPLC with a Luna 5 □ RP-C18(2) semipreparative column (250 X 10.0 mm; Phenomenex, Torrance, CA) and using the following conditions: flow rate of 2 mL/min; 0.05% trifluoroacetate (TFA)-CH_3CN from 100:0 to 95:5 (System A) (or up to 99:1 to 50:50 (System B)) in 30 min (and isolated as the TFA salt form). Prior to testing for biological activity, compound **6** was shown to be >98% pure by HPLC analysis (detection at 254 nm). All other compounds were purified only by silica column.

Ethyl-7-(4-(trifluoromethyl)-phenyl)-4-hydroxy-2-naphthoate (2)

Ethyl-7-bromo-4-hydroxy-2-naphthoate (**1**) (0.70 g, 2.37 mmol), 4-(trifluoromethyl)phenylboronic acid (0.5 g, 2.63 mmol), 2 M Na_2CO_3 (3.6 mL, 7.17 mmol) and DMF (6 mL) were added to a 25 mL round bottom flask under N_2. This suspension was degassed three times, and solid $PdCl_2$(dppf) (0.035 g, 0.047 mmol) was added to

the reaction flask. The starting material disappeared after 2.5 h reaction at 85 ^{o}C. Then DMF was removed and the residue re-suspended in 50 mL EtOAc. The inorganic byproducts were removed by partitioning with water followed by extraction (3X) with EtOAc. The combined organic layer was then washed with brine, dried with Na_2SO_4 and evaporated. The resulting crude mixture was purified with silica column eluting with 20% EtOAc: hexane to obtain **2** (1.9 mmol, 80% yield). ^{1}H NMR (Acetone-d_6) δ: 9.51(s, 1H), 8.41 (s, 1H), 8.40 (d, J = 5.8 Hz, 1H), 8.26 (s, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.95 (dd, J_1 =8.8 Hz J_2 = 1.8 Hz, 1H), 7.87 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 1.4 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H); HRMS-EI found 359.1 $(M - H^{+})^{-}$.

Ethyl-7-(4-(trifluoromethyl)phenyl)-4-(((trifluoromethyl)sulfonyl)oxy)-2-naphthoate (3)

Compound **1** (0.154 g, 0.40 mmol) was added to a 25 mL round bottom flask containing pyridine (0.048 mL, 0.6 mmol) and 2 mL of CH_2Cl_2. This suspension was cooled to -78 ^{o}C and treated with trifluoromethanesulfonic anhydride (0.078 mL, 0.460 mmol). The reaction flask was then warmed to room temperature and stirred for 4 h. After the reaction was judged complete by TLC, an additional 20 mL of CH_2Cl_2 was added to the flask. This organic phase was washed successively with 25 mL of 10% $NaHCO_3$, 25 mL of 1 N HCl and brine. Finally, the crude organic phase was dried with Na_2SO_4 and solvent evaporated. The resulting crude product was rinsed with hexane and subjected to the next reaction without further purification.

Ethyl-7-(4-(trifluoromethyl)phenyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-naphthoate (4)

Compound **3** (approx. 0.4 mmol), bis(pinacolato)diboron (0.111 g, 0.440 mmol), potassium acetate (0.118 g, 1.2 mmol) and 2.5 mL of dioxane were added to a 25 mL

round bottom flask under N_2. The reaction mixture was degassed and then treated with solid $PdCl_2$(dppf) (0.0082 g, 0.01 mmol). The resulting suspension was then warmed to 85 ^oC for 5 h. After disappearance of the starting materials, solvent was evaporated and the pure product **4** (0.117 mg, 0.25 mmol, 63% yield) was obtained after the column chromatography (silica gel, mobile phase of 20% EtOAc: hexane). ^1H NMR ($CDCl_3$) δ: 8.30 (d, *J* = 8.6 Hz, 1H), 8.25 (s, 1H), 8.00 (s, 1H), 7.87 (m, 3H), 7.72 (m, 3H), 7.43 (d, *J* = 1.3 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.22 (s, 12H); m/z (M+ESI MS) found: 471.1949; calc for $C_{26}H_{27}B_{11}O_4F$: 471.1954.

Ethyl-4-(((4-*tert*-butylcarbonyl)-3,6-dihydro-2H-pyridinyl)phenyl)-(7-(4 (trifluoromethyl)-phenyl)-2-naphthoate (5)

Compound **4** (25.0 mg, 0.053 mmol), 4-(4-bromophenyl)-3,6-dihydro-2H-pyridine-1-carboxylic acid *tert*-butyl ester (27.2 mg, 0.079 mmol), K_2CO_3 (22.1 mg, 0.159 mmol) and DMF (0.5 mL) were added to a 25 mL round bottom flask under N_2. This suspension was degassed three times, and solid $Pd(PPh_3)_4$ (12 mg, 0.010 mmol) was added to the reaction flask. The starting material disappeared after 2.0 h at 110 ^oC. After cooling, DMF was removed by evaporation in vacuo, and the resulting crude mixture was purified on a silica column eluting with 20% EtOAc: hexane to obtain **5** (22 mg, 0.036 mmol, 70% yield). ^1H NMR ($CDCl_3$) δ: 8.65 (s, 1H), 8.20 (s, 1H), 8.03 (s, 1H), 8.02 (d, *J* = 6.7 Hz , 1H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.76 (d, *J* = 1.8 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.52 (d, *J* = 8.4 Hz, 2H), 7.58 (d, *J* = 6.5 Hz, 1H), 7.44 (d, *J* = 8.3 Hz, 2H), 6.10 (s (broad),1H), 4.43 (q, *J* = 7.1 Hz, 2H), 4.11(m, 2H), 3.67(t, *J* = 5.4 Hz, 2H), 2.36 (m, 2H), 1.5 (s, 9H) 1.42 (t, *J* = 7.1 Hz, 3H).

Ethyl-4-(((4-*tert*-butylcarbonyl)-piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-

phenyl)-2-naphthoate

Compound **5** (44 mg, 0.07 mmol), Rh/C (44 mg) and EtOAc (3 mL) were placed in a 10 mL flask. This mixture was hydrogenated at 100 psi in a Parr apparatus for overnight at room temperature. The mixture was then filtered through a pad of Celite, and the filtrate was concentrated to obtain the named product. This product was subjected to next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ: 8.64 (s, 1H), 8.20 (s, 1H), 8.03 (m, 2H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.41 (q, *J* = 7.1 Hz, 2H), 2.83(m, 2H), 2.27(m, 2H), 1.90 (m, 2H), 1.69 (m, 2H), 1.4 (s, 9H) 1.42 (t, *J* = 7.1 Hz, 3H).

4-(((4-*tert*-Butylcarbonyl)piperidin-4-yl) phenyl)-(7-(4 (trifluoromethyl)-phenyl)-2-naphthoic acid

The crude solid product from the above reaction was added to 25 mL seal tube and treated with 0.5 mL of THF:DMSO:MeOH (1:1:1) mixture. Then, 4 M LiOH (0.122 mL, 0.49 mmol) was added and the tube sealed with a Teflon cap. The reaction mixture was heat at 80 ^{o}C for overnight. Then mixture was cooled to room temperature, treated with 1 N HCl until pH < 2 was reached and extracted three times (5 mL) with EtOAc. The organic phase was then dried and product was purified using a silica column (30% methanol: $CHCl_3$) to get the named naphthoic acid derivative (0.032 g, 0.055 mmol, 78% for 2 steps). m/z HRMS-AP found: 574.2188; calc for $C_{34}H_{31}NO_4F_3$: 574.2205.

4-((Piperidin-4-yl)-phenyl)-(7-(4-(trifluoromethyl)-phenyl)-2-naphthoic acid (PPTN) (6)

The naphthoic acid derivative from the above reaction (0.032 g, 0.055 mmol) was dissolved in 1 mL CH_2Cl_2 in a 10 mL round bottom flask. Then TFA (10 eq, 0.055 mL, 0.55 mmol) was added at room temperature and the mixture stirred for 2 h. Then the

solvent was removed, and the deprotected product (**6**) was isolated using HPLC purification (mobile phase 0.05% TFA-CH_3CN). ^1H NMR (D_2O) δ: 8.71 (s, 1H), 8.39 (s, 1H), 7.95 (m, 4H), 7.89 (dd, J_1 = 8.8 Hz J_2 = 1.8 Hz, 1H), 7.77 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 3.63 (m, 1H), 3.53 (m, 2H), 3.01 (m, 1H), 2.15 (m, 2H), 1.99 (m, 2H); HRMS-EI found: 474.1658 $(M - H^+)^-$. $C_{29}H_{23}NO_2F_3$ requires 474.1681; (M+ESI MS) found: 476.1826; calc for $C_{29}H_{25}NO_2F_3$ $(M + H^+)$: 476.1837; purity > 95% by HPLC.

Abbreviations

DMSO, dimethylsulfoxide

EtOAc, ethyl acetate

EI, electron ionization

ESI, electrospray ionization

HPLC, high performance liquid chromatography

HRMS, high resolution mass spectrum

NMR, nuclear magnetic resonance

TBAP, tetrabutylammonium dihydrogen phosphate

TFA, trifluoroacetic acid

THR, tetrahydrofuran

References

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