Supplementary Materials

Selection, Preparation, and Evaluation of Small-Molecule Inhibitors of Toll-Like Receptor 4

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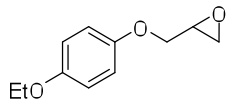
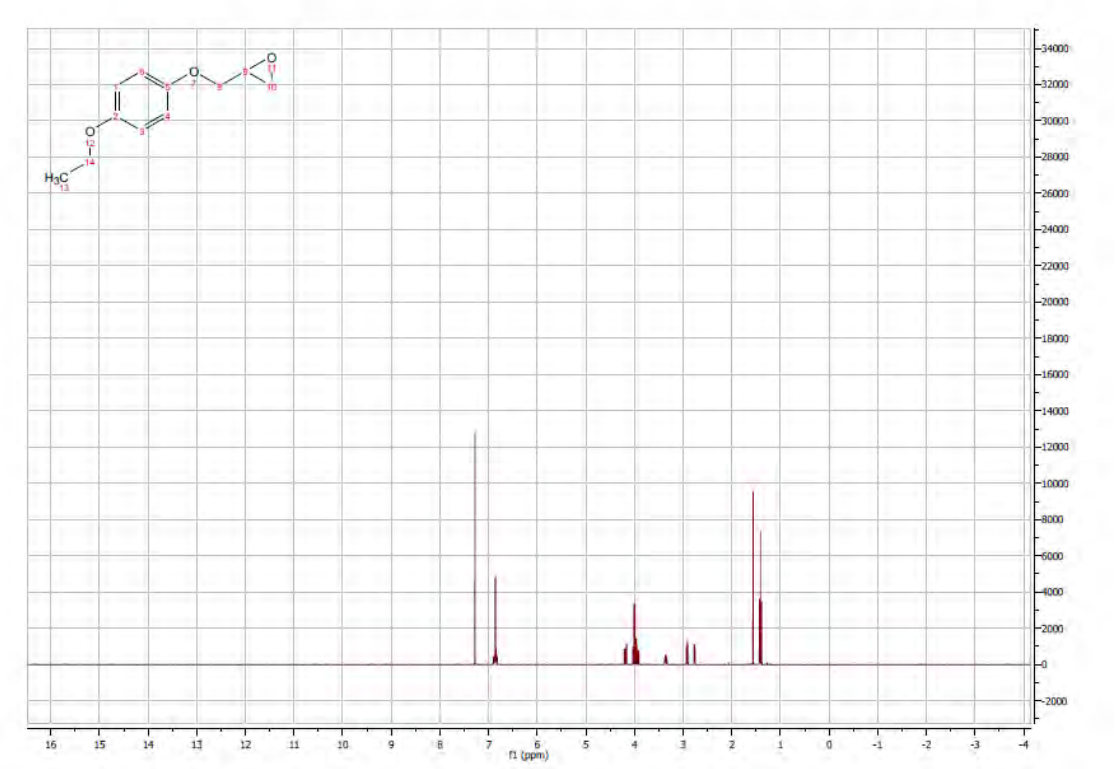
General Information

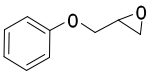
All reactions were run in oven-dried or flame-dried glassware under a dry nitrogen or argon atmosphere. Methanol and ethanol were distilled by simple distillation and stored over 4Å molecular sieves. Acetone was distilled before use by simple distillation. Methylamine.HCl salt and powdered potassium hydroxide were dried under high-vac overnight using P_2O_5 as a decadent. All other reagents and solvents were used as received from the supplier. Flash chromatography was performed using 32-64 μm silica gel. ^{1}H NMR spectra were recorded at either 300 MHz, 400 MHz, or 500 MHz in $CDCl_3$ using residual $CHCl_3$ (7.26 ppm) as the internal standard. ^{13}C NMR spectra were recorded at 75 MHz in $CDCl_3$ using residual $CHCl_3$ (77.23 ppm) as an internal reference. Exact mass was determined using an electrospray (ESI)-triple quadrupole-time-of-flight (TOF) mass spectrometer from Applied Biosystems, PE SCIEX/ABI API QSTAR Pulsar i Hybrid LC/MS/MS . Enantiomeric excess was determined by ChiralPak AD HPLC column or optical rotation done on a Jasco P-1030 polarimeter.



2-((4-ethoxyphenoxy)methyl)oxirane (3)

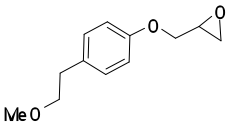
General Procedure: 4-Ethoxyphenol (**5,** 0.250 g, 1.81 mmol), anhydrous potassium carbonate (0.500 g, 3.62 mmol) and epichlorohydrin (**4,** 0.570 mL, 7.24 mmol) were added to acetone (4.52 mL) and the resulting heterogeneous solution was refluxed for 20 h. The mixture was cooled to room temperature and filtered through a pad of celite and the filtrate was concentrated under reduced pressure. The resulting oil was dissolved in toluene (20 mL), washed with sequentially with water (15 mL), 5% aqueous NaOH (20 mL), and water again (20 mL) before being dried with $MgSO_4$ and concentrated under reduced pressure. 0.292g (83%) of 2-((4-ethoxyphenoxy)methyl)oxirane as a white solid (mp= 41 °C) was isolated using column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 – 6.77 (m, 4H), 4.17 (dd, *J* = 11.0, 3.2, 1H), 3.98 (q, *J* = 7.0, 2H), 3.91 (dd, *J* = 11.0, 5.6, 1H), 3.34 (m, 1H), 2.90 (dd, *J* = 4.9, 4.1, 1H), 2.75 (dd, *J* = 5.0, 2.7, 1H), 1.39 (t, *J*=6.98, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.72, 152.78, 115.90, 115.90, 115.59, 115.59, 69.71, 64.18, 50.49, 44.98, 15.15. HRMS (*m/z*): [MNa]+ calc. for $C_{11}H_{14}O_3Na$+, 217.08; found 217.0826.





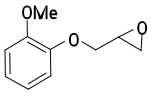
2-(phenoxymethyl)oxirane

Used general procedure from 2-((4-ethoxyphenoxy)methyl)oxirane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.23 (m, 2H), 7.03 – 6.87 (m, 3H), 4.22 (dd, *J* = 11.0, 3.3, 1H), 3.97 (dd, *J* = 11.0, 5.6, 1H), 3.43 – 3.31 (m, 1H), 2.91 (dd, *J* = 4.9, 4.1, 1H), 2.77 (dd, *J* = 4.9, 2.7, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.44, 129.51, 129.51, 121.22, 114.60, 114.60, 68.65, 50.17, 44.77. HRMS (*m/z*): [MLi]+ calc. for $C_9H_{10}O_2Li$+, 157.11, found 157.0840.



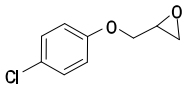
2-((4-(2-methoxyethyl)phenoxy)methyl)oxirane

Used general procedure from 2-((4-ethoxyphenoxy)methyl)oxirane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 – 7.10 (m, 2H), 6.90 – 6.82 (m, 2H), 4.22 – 4.16 (dd, *J*= 9.0, 3.0, 1H), 3.95 (dd, *J* = 11.0, 5.6, 1H), 3.56 (t, *J* = 7.1, 2H), 3.33 (s, 3H), 2.90 (dd, *J* = 4.9, 4.1, 1H), 2.82 (t, *J* = 7.1, 2H), 2.75 (dd, *J* = 4.9, 2.7, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.97, 131.64, 129.81, 129.80, 114.58, 114.50, 73.81, 68.79, 58.67, 50.19, 44.77, 35.30. HRMS (*m/z*): [MLi]+ calc. for $C_{12}H_{16}O_3Li$+ 215.05, found 215.1250.



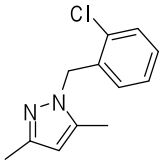
2-((2-methoxyphenoxy)methyl)oxirane

Used general procedure from 2-((4-ethoxyphenoxy)methyl)oxirane. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 – 6.86 (m, 4H), 4.24 (dd, *J* = 11.4, 3.6, 1H), 4.08 – 4.01 (m, 1H), 3.93 – 3.85 (m, 3H), 3.43 – 3.34 (m, 1H), 2.90 (t, *J* = 4.5, 1H), 2.75 (dd, *J* = 4.9, 2.6, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.65, 147.99, 121.96, 120.86, 114.27, 111.95, 70.24, 55.89, 50.23, 45.02. HRMS (*m/z*): [MLi]+ calc. for $C_{10}H_{12}O_3Li$+ 187.02, found 187.0948.



2-((4-chlorophenoxy)methyl)oxirane

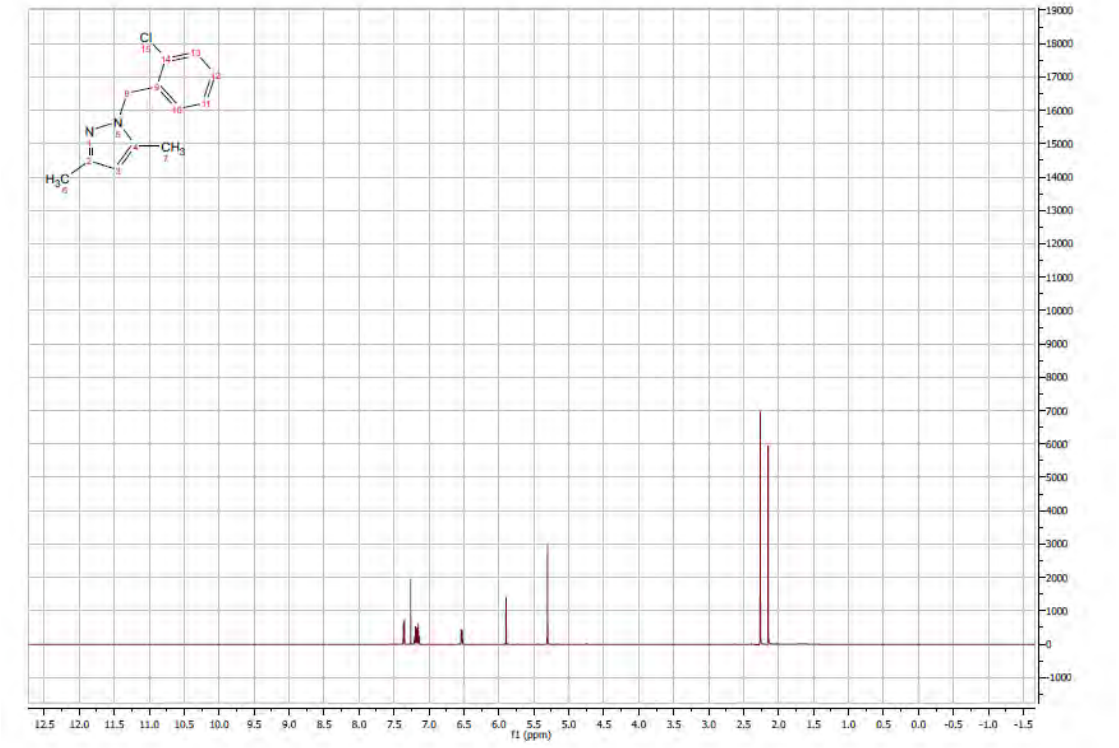
Used general procedure from 2-((4-ethoxyphenoxy)methyl)oxirane. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 – 7.15 (m, 2H), 6.93 – 6.79 (m, 1H), 4.21 (dd, *J* = 11.0, 3.0, 1H), 3.91 (dd, *J* = 11.0, 5.7, 1H), 3.35 (ddt, *J* = 5.7, 4.1, 2.8, 1H), 2.91 (dd, *J* = 4.9, 4.2, 1H), 2.75 (dd, *J* = 4.9, 2.7, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.07, 129.38, 129.38, 126.14, 115.93, 115.93, 69.05, 50.04, 44.62. HRMS (*m/z*): [MLi]+ calc. for $C_9H_9ClO_2Li$+ 190.97, found 191.0449.

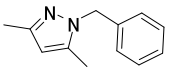


1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole (8)

General Procedure: Powdered potassium hydroxide (1.751 g, 31.20 mmol) was added to a solution of 3,5-dimethylpyrazole (**6**, 2.000 g, 20.81 mmol) in anhydrous DMSO (10.40 mL) and the resulting heterogeneous solution was stirred for 1.5 h at 80 °C before being cooled to room temperature. 2-chloro benzylchloride (**7,** 2.64 mL, 20.8 mmol) was then added as a 6 M solution in DMSO over 15 min., and the reaction mixture was stirred for a further 1.5 h. Upon completion as observed by TLC, the reaction was poured over water, and the resulting aqueous phase was extracted with two 20 mL portions of $CHCl_3$. The combined organic layers were washed with 100 mL of water, dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. 4.55 g (99%) of 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole was isolated as a clear liquid and required no further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ

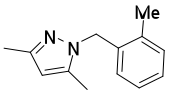
7.41 – 7.31 (m, 1H), 7.24 – 7.09 (m, 2H), 6.59 – 6.50 (m, 1H), 5.90 (s, 1H), 5.31 (s, 2H), 2.26 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.32, 139.96, 135.46, 131.96, 129.42, 128.76, 127.72, 127.48, 105.84, 50.12, 13.80, 11.15. HRMS (*m/z*): [MNa]+ calc for $C_{12}H_{13}ClN_2Na$+ 243.07; found 243.0651.





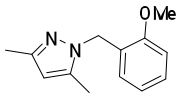
1-benzyl-3,5-dimethyl-1H-pyrazole

Used general procedure from 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 – 7.28 (m, 2H), 7.26 – 7.22 (m, 1H), 7.07 (ddd, *J* = 5.3, 1.3, 0.6, 2H), 5.85 (s, 1H), 5.22 (s, 2H), 2.25 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.54, 139.21, 137.39, 128.67, 128.67, 127.41, 126.57, 126.57, 105.55, 52.62, 13.54, 11.14. HRMS (*m/z*): [MNa]+ calc. for $C_{12}H_{14}N_2Na$+ 209.11, found 209.1047.



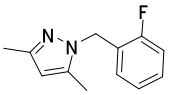
3,5-dimethyl-1-(2-methylbenzyl)-1H-pyrazole

Used general procedure from 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 5.1, 1.2, 2H), 7.13 – 7.05 (m, 1H), 6.48 (d, *J* = 7.3, 1H), 5.88 (s, 1H), 5.19 (s, 1H), 2.33 (s, 3H), 2.26 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.08, 147.66, 134.69, 130.09, 127.21, 126.38, 125.84, 105.43, 50.54, 19.08, 13.56, 11.08. HRMS (*m/z*): [MH]+ calc. for $C_{13}H_{17}N_2$+ 201.13, found 201.1392.



1-(2-methoxybenzyl)-3,5-dimethyl-1H-pyrazole

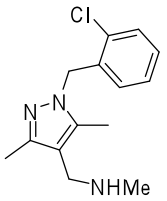
Used general procedure from 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.15 (m, 1H), 6.91 – 6.79 (m, 2H), 6.59 (ddt, *J* = 7.6, 1.6, 0.9, 1H), 5.84 (s, 1H), 5.21 (s, 1H), 3.85 (s, 3H), 2.24 (s, 3H), 2.16 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.11, 147.57, 139.60, 128.33, 127.30, 125.95, 120.78, 109.87, 105.16, 55.29, 47.26, 13.59, 10.92. HRMS (*m/z*): [MH+] calc for $C_{13}H_{17}N_2O$+ 217.13, found 217.1328.



1-(2-fluorobenzyl)-3,5-dimethyl-1H-pyrazole

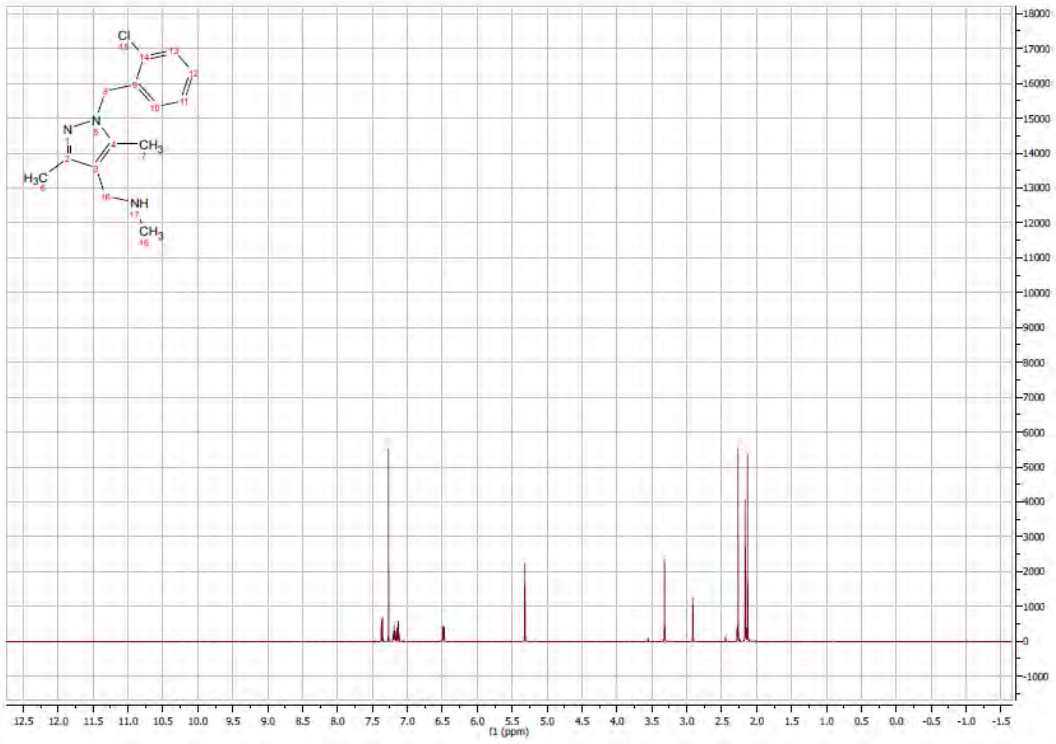
Used general procedure from 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.18 (m, 1H), 7.11 – 6.99 (m, 2H), 6.86 (t, *J* = 6.9, 1H), 5.86 (s, 1H),

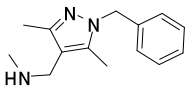
5.27 (s, 2H), 2.26 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.21, 129.11, 128.78, 124.53, 124.49, 115.26, 114.98, 105.57, 45.96, 45.89, 13.53, 10.89. HRMS (*m/z*): [MNa+] calc for $C_{12}H_{13}FN_2Na$+ 227.10, found 227.0951.



1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine (3)

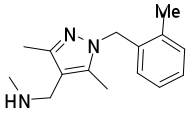
General Procedure: A solution of paraformaldehyde (0.820 g, 27.2 mmol) and methylamine.HCl (0.920 g, 13.6 mmol) was dissolved in ethanol (9.06 mL) was stirred for 1h, then 1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazole (**8**, 1.00 g, 4.53 mmol) was added and the reaction mixture was stirred at reflux for 16 h. The mixture was then cooled to room temperature and quenched with aqueous $NaHCO_3$ (15 mL). The aqueous layer was extracted 3 times with chloroform (15 mL) and the combined organic layers were washed with brine (30 mL). The organic layer was dried with $MgSO_4$ and concentrated under reduced pressure. The resulting yellow oil was purified used flash column chromatography with 3% methanol in dichloromethane as an eluting solvent to obtain the product in a 93% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 – 7.31 (m, 1H), 7.23 – 7.08 (m, 2H), 6.54 – 6.43 (m, 1H), 5.32 (s, 2H), 3.31 (s, 2H), 2.91 (s, 1H), 2.25 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.12, 138.48, 135.57, 131.94, 129.42, 128.72, 127.61, 127.41, 114.38, 50.24, 49.08, 40.68, 12.36, 9.75. HRMS (*m/z*): [MH]+ calc for $C_{14}H_{18}ClN_3$, 264.13; found 264.1253.





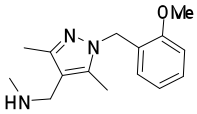
1-(1-benzyl-3,5-dimethyl-1H-pyrazol-4-yl)-N-methylmethanamine

Used general procedure from 1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29-7.26 (m, 2H), 7.24-7.21 (m, 1H), 7.05 – 7.01 (m, 2H), 5.22 (s, 2H), 3.27 (s, 2H), 2.87 (s, 1H), 2.24 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.34, 137.54, 128.65, 128.65, 127.36, 126.46, 126.46, 114.09, 79.94, 52.69, 48.84, 40.42, 12.08, 9.74. HRMS (*m/z*): [MH]+ calc. for $C_{14}H_{20}N_3$+ 230.17, found 230.1655.



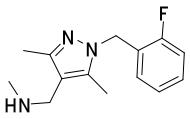
1-(3,5-dimethyl-1-(2-methylbenzyl)-1H-pyrazol-4-yl)-N-methylmethanamine

Used general procedure from 1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (dt, *J* = 8.0, 3.3, 2H), 7.11 – 7.00 (m, 1H), 6.42 (d, *J* = 7.4, 1H), 5.20 (s, 2H), 3.30 (s, 1H), 2.33 (s, 3H), 2.25 (s, 3H), 2.09 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.48, 138.23, 135.72, 134.63, 130.08, 127.16, 126.34, 125.70, 79.82, 50.64, 48.88, 40.47, 19.08, 12.13, 9.68. HRMS (*m/z*): [MNa]+ calc. for $C_{15}H_{21}N_3Na$+ 266.16, found 266.2638.



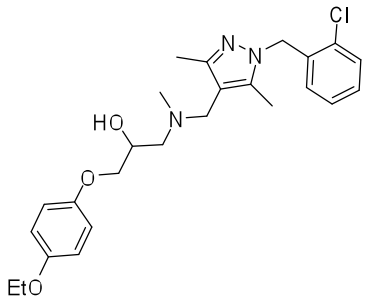
1-(1-(2-methoxybenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)-N-methylmethanamine

Used general procedure from 1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (td, *J* = 8.2, 1.7, 1H), 6.89 – 6.77 (m, 2H), 6.51 (dd, *J* = 7.5, 1.4, 1H), 5.20 (s, 2H), 3.86 (s, 3H), 3.30 (s, 1H), 2.24 (s, 3H), 2.14 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.08, 147.39, 138.22, 128.25, 127.08, 126.10, 120.74, 109.84, 54.09, 48.91, 47.38, 40.46, 14.20, 12.15, 9.53. HRMS (*m/z*): [MNa+] calc for $C_{15}H_{21}N_3ONa$+ 282.16, found 282.1564.



1-(1-(2-fluorobenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)-N-methylmethanamine

Used general procedure from 1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 – 7.14 (m, 1H), 7.06 – 6.98 (m, 2H), 6.77 (td, *J* = 7.5, 1.5, 1H), 5.26 (s, 2H), 3.26 (s, 2H), 2.88 (s, 1H), 2.21 (s, 3H), 2.13 (s, 3H), 2.12 (s, 3H). HRMS (*m/z*): [MH+] calc for $C_{14}H_{19}FN_3$+ 248.15, found 248.1559.



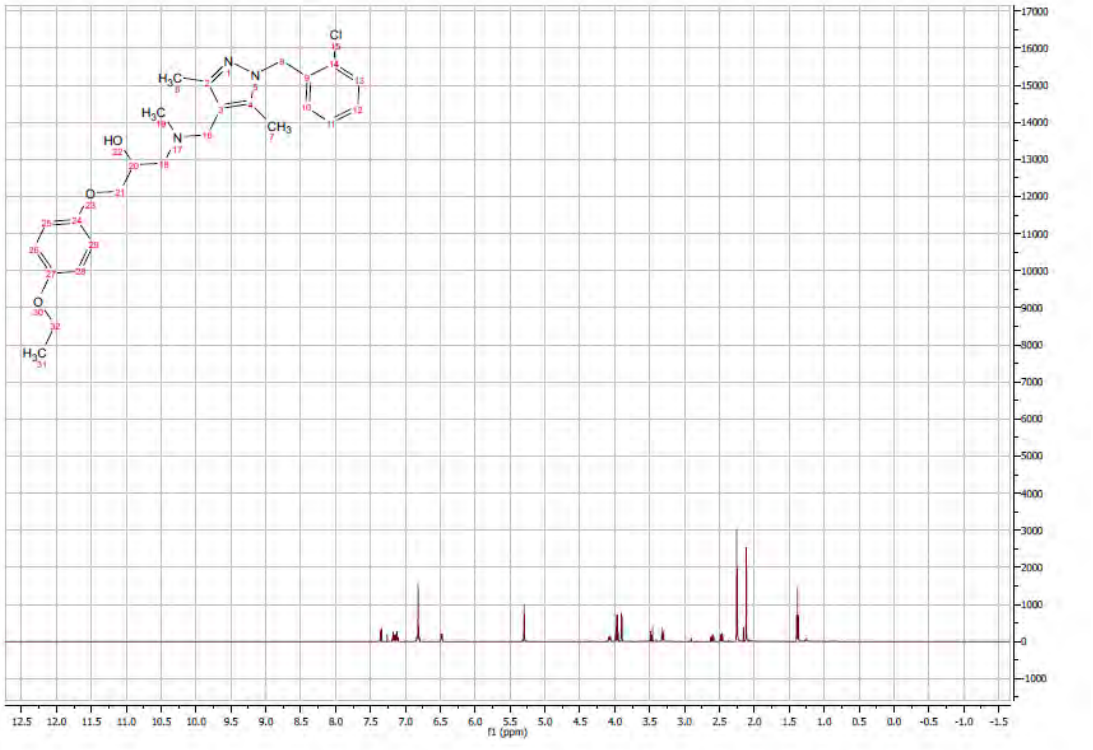
1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol (1)

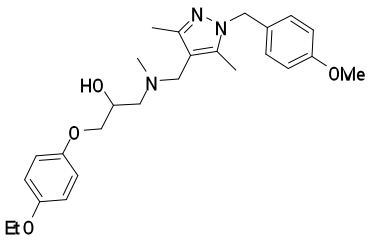
2-((4-Ethoxyphenoxy)methyl)oxirane (**6,** 0.060 g, 0.32 mmol) and 1-(1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)-*N*-methylmethanamine (**3,** 0.10 g, 0.38 mmol) were dissolved in ethanol (0.19 mL) and warmed to 75 °C and stirred until the oxirane was totally consumed as observed by TLC (20-24 h). The solution was then cooled to room temperature and diluted with chloroform. The organic phase was washed with sat'd sodium bicarbonate, and the organic layer was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The resulting oil was purified using flash column chromatography with 2% methanol in dichloromentane as an eluting solvent, yielding 0.09g (89%) of 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol as a clear

liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 7.8, 1.2, 1H), 7.17 (td, *J* = 7.7, 1.3, 1H), 7.12 (td, *J* = 7.5, 1.2, 1H), 6.85 – 6.79 (m, 4H), 6.48 (dd, *J* = 7.6, 0.9, 1H), 5.28 (s, 2H), 4.12 – 4.04 (m, 1H), 3.97 (q, *J* = 7.0, 2H), 3.90 (d, *J* = 4.9, 2H), 3.47 (d, *J* = 13.2, 1H), 3.34 – 3.29 (m, 1H), 2.60 (dd, *J* = 12.2, 9.7, 1H), 2.48 (dq, *J* = 12.2, 3.9, 1H), 2.26 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.38 (t, *J* = 9.1, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.47, 153.04, 148.04, 138.67, 135.31, 131.92, 129.43, 128.79, 127.58, 127.50, 115.63, 115.63, 115.53, 115.53, 113.71, 71.25, 66.37, 64.14, 59.51, 51.70, 50.28, 42.09, 15.13, 12.36, 9.82. HRMS (*m/z*): [MNa]+ calc for $C_{25}H_{32}ClN_3O_3Na$+, 480.20; found 480.2030.

(+)-(S)-1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol [α_D] +17.6, *calc.* 99% *ee*

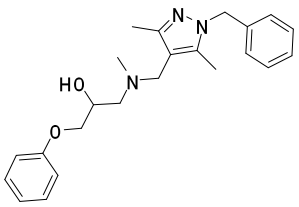
(-)-(R)-1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol [α_D] -17.7, 99% *ee* by chiral HPLC





1-(4-ethoxyphenoxy)-3-(((1-(4-methoxybenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)propan-2-ol (11)

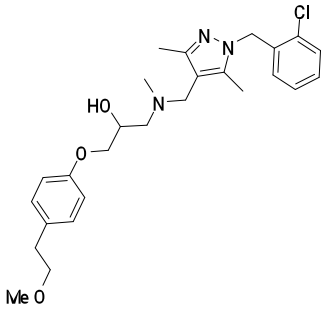
Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (dd, *J* = 6.9, 4.8, 2H), 6.87 – 6.77 (m, 6H), 5.15 (s, 2H), 4.06 (td, *J* = 9.3, 4.8, 1H), 3.97 (q, *J* = 7.0, 2H), 3.89 (d, *J* = 5.0, 2H), 3.76 (s, 3H), 3.44 (d, *J* = 13.2, 1H), 3.28 (d, *J* = 13.2, 1H), 2.59 (dd, *J* = 12.2, 9.8, 1H), 2.45 (dd, *J* = 12.2, 4.1, 1H), 2.24 (s, 3H), 2.22 (s, 3H), 2.12 (s, 3H), 1.38 (t, *J* = 7.0, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.94, 153.28, 152.85, 147.15, 137.85, 129.34, 127.89, 127.89, 115.44, 115.44, 115.35, 115.35, 114.08, 114.08, 113.14, 71.07, 66.10, 63.96, 59.37, 55.23, 52.35, 51.48, 41.79, 14.94, 12.14, 9.89. HRMS (*m/z*): [MLi+] calc for $C_{26}H_{35}N_3O_4Li$+ 460.20, found 460.2792.



1-(((1-benzyl-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)-3-phenoxypropan-2-ol (12)

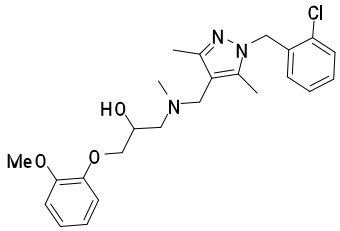
Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ

7.33 – 7.20 (m, 5H), 7.10 – 7.03 (m, 2H), 6.98 (dd, *J* = 4.7, 3.7, 1H), 6.95 – 6.89 (m, 2H), 5.23 (s, 2H), 4.17 – 4.04 (m, 1H), 3.96 (dd, *J* = 5.0, 0.8, 1H), 3.47 (d, *J* = 13.2, 1H), 3.31 (d, *J* = 13.2, 1H), 2.62 (dd, *J* = 12.2, 9.6, 1H), 2.53 – 2.45 (m, 1H), 2.26 (s, 3H), 2.25 (s, 3H), 2.13 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.72, 147.30, 138.02, 137.30, 129.45, 129.45, 128.72, 128.72, 127.47, 126.48, 126.48, 120.94, 114.51, 114.51, 113.29, 70.28, 66.05, 59.37, 52.81, 51.51, 41.82, 12.14, 9.85. HRMS (*m/z*): [MH]+ calc. for $C_{23}H_{30}N_3O_2$+ 380.24, found 380.2326.



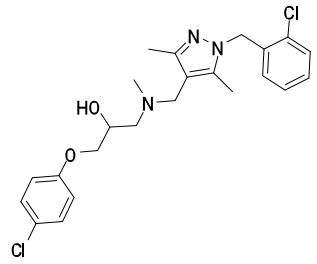
1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-(2-methoxyethyl)phenoxy)propan-2-ol (13)

Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 7.7, 1.5, 1H), 7.21 – 7.09 (m, 4H), 6.88 – 6.80 (m, 2H), 6.52 – 6.45 (m, 1H), 5.30 (s, 2H), 4.18 – 4.03 (m, 1H), 3.96 – 3.90 (m, 2H), 3.56 (t, *J* = 8.9, 2H), 3.49 (d, *J* = 13.2, 1H), 3.35 (s, 3H), 3.32 (d, *J* = 13.2, 1H), 2.82 (t, *J* = 7.1, 2H), 2.62 (dd, *J* = 12.2, 9.6, 1H), 2.48 (dd, *J* = 12.3, 4.2, 1H), 2.26 (s, 6H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.24, 147.87, 138.51, 135.12, 131.75, 131.33, 129.93, 129.76, 129.76, 129.25, 128.61, 127.40, 127.32, 114.45, 114.45, 73.85, 70.38, 66.08, 59.35, 58.66, 51.51, 50.11, 41.88, 35.29, 12.18, 9.65. HRMS (*m/z*): [MH]+ calc. for $C_{26}H_{34}ClN_3O_3H$+ 472.23, found 472.2354.



1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)-3-(2-methoxyphenoxy)propan-2-ol (14)

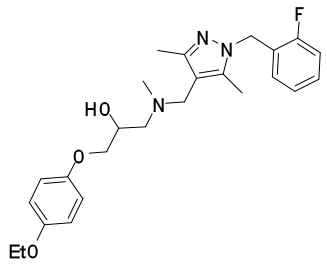
Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (dd, *J* = 7.8, 1.4, 1H), 7.15 (dtd, *J* = 16.5, 7.4, 1.7, 2H), 6.99 – 6.84 (m, 4H), 6.51 – 6.46 (m, 1H), 5.30 (s, 2H), 4.22 – 4.09 (m, 1H), 4.02 – 3.98 (m, 2H), 3.85 (s, 3H), 3.47 (d, *J* = 13.2, 1H), 3.33 (d, *J* = 13.2, 1H), 2.57 (qd, *J* = 12.4, 6.9, 2H), 2.26 (s, 6H), 2.12 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.79, 148.33, 147.90, 145.21, 138.55, 135.13, 131.73, 129.24, 128.59, 127.40, 127.30, 121.78, 120.90, 114.51, 111.94, 72.33, 66.28, 59.44, 55.84, 51.54, 50.10, 41.95, 12.17, 9.64. HRMS (*m/z*): [MH]+ calc. for $C_{24}H_{30}ClN_3O_3H$+ 444.20, found 444.2046.



1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-chlorophenoxy)propan-2-ol (15)

Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ

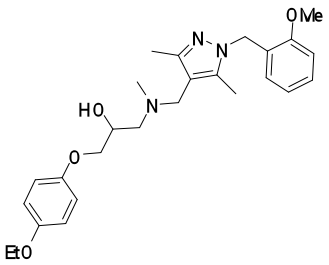
7.38 (dd, *J* = 7.8, 1.4, 1H), 7.27 – 7.22 (m, 2H), 7.22 – 7.17 (m, 1H), 7.14 (td, *J* = 7.5, 1.6, 1H), 6.90 – 6.80 (m, 2H), 6.52 (d, *J* = 9.1, 1H), 5.32 (s, 2H), 4.19 – 4.07 (m, 1H), 3.94 (d, *J* = 4.9, 2H), 3.54 (d, *J* = 13.0, 1H), 3.37 (d, *J* = 13.2, 1H), 2.66 (t, *J* = 10.8, 1H), 2.51 (d, *J* = 10.8, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.37, 147.83, 138.46, 135.09, 131.77, 129.31, 129.31, 129.27, 128.62, 127.40, 127.29, 125.84, 115.80, 115.08, 113.41, 70.68, 65.97, 59.10, 51.51, 50.12, 41.89, 12.18, 9.65. HRMS (*m/z*): [MH]+ calc. for $C_{23}H_{28}Cl_2N_3O_2$+ 448.15, found 448.1566.



1-(4-ethoxyphenoxy)-3-(((1-(2-fluorobenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)propan-2-ol (16)

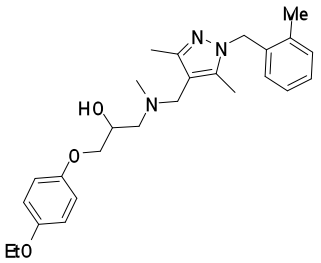
Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (ddd, *J* = 7.1, 4.5, 1.8, 1H), 7.04 (ddd, *J* = 14.2, 6.5, 4.3, 2H), 6.86 – 6.75 (m, 5H), 5.26 (s, 2H), 4.07 (dt, *J* = 14.3, 4.6, 1H), 3.97 (q, *J* = 7.0, 2H), 3.90 (d, *J* = 5.0, 2H), 3.46 (d, *J* = 13.2, 1H), 3.29 (d, *J* = 13.2, 1H), 2.60 (dd, *J* = 12.2, 9.6, 1H), 2.51 – 2.42 (m, 1H), 2.24 (s, 3H), 2.22 (d, 3H), 2.15 (s, 3H), 1.38 (t, *J* = 7.0, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.28, 152.86, 147.68, 138.22, 129.23, 129.12, 128.59, 128.54, 124.56, 115.44, 115.44, 115.34,

115.34, 115.02, 71.06, 66.14, 63.97, 59.39, 51.50, 46.19, 46.12, 41.82, 14.94, 12.17, 9.60. HRMS (*m/z*): [MH+] calc for $C_{25}H_{33}FN_3O_3$+ 442.24, found 442.2515.



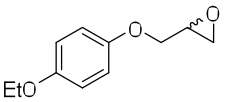
1-(4-ethoxyphenoxy)-3-(((1-(2-methoxybenzyl)-3,5-dimethyl-1H-pyrazol-4-yl)methyl)(methyl)amino)propan-2-ol (17)

Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 – 7.13 (m, 1H), 6.91 – 6.75 (m, 6H), 6.54 (dd, *J* = 7.5, 1.6, 1H), 5.21 (s, 2H), 4.08 (dd, *J* = 9.5, 4.4, 1H), 3.97 (q, *J* = 7.0, 2H), 3.90 (d, *J* = 5.0, 2H), 3.85 (s, 3H), 3.47 (d, *J* = 13.2, 1H), 3.30 (d, *J* = 13.2, 1H), 2.60 (dd, *J* = 12.2, 9.7, 1H), 2.51 – 2.43 (m, 1H), 2.24 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.38 (t, *J* = 7.0, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.10, 153.26, 152.89, 147.32, 138.39, 128.38, 127.16, 125.84, 120.80, 115.46, 115.46, 115.34, 115.34, 112.96, 109.91, 71.14, 66.14, 63.96, 59.39, 55.29, 51.55, 47.47, 41.79, 14.94, 12.19, 9.63. HRMS (*m/z*): [MH+] calc for $C_{26}H_{34}N_3O_4$+ 454.26, found 454.2700.



1-(((3,5-dimethyl-1-(2-methylbenzyl)-1H-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol (18)

Used general procedure from 1-(((1-(2-chlorobenzyl)-3,5-dimethyl-1*H*-pyrazol-4-yl)methyl)(methyl)amino)-3-(4-ethoxyphenoxy)propan-2-ol. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 – 7.13 (m, 2H), 7.13 – 7.02 (m, 1H), 6.88 – 6.78 (m, 4H), 6.43 (d, *J* = 7.4, 1H), 5.19 (s, 2H), 4.08 (tt, *J* = 9.9, 4.9, 1H), 3.98 (q, *J* = 7.0, 2H), 3.91 (d, *J* = 5.0, 2H), 3.49 (d, *J* = 13.2, 1H), 3.32 (d, *J* = 13.2, 1H), 2.61 (dd, *J* = 12.2, 9.6, 1H), 2.52 – 2.44 (m, 1H), 2.32 (s, 3H), 2.25 (s, 3H), 2.10 (s, 3H), 1.39 (t, *J* = 7.0, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.28, 152.87, 147.42, 138.41, 135.48, 134.66, 130.14, 127.25, 126.43, 125.69, 115.45, 115.45, 115.34, 115.34, 113.16, 71.08, 66.12, 63.96, 59.37, 51.52, 50.74, 41.82, 19.09, 14.94, 12.18, 9.78. HRMS (*m/z*): [MNa]+ calc. for $C_{26}H_{35}N_3O_3Na$+ 460.26, found 460.2586.



(+) and (-) 2-((4-ethoxyphenoxy)methyl)oxirane (3)

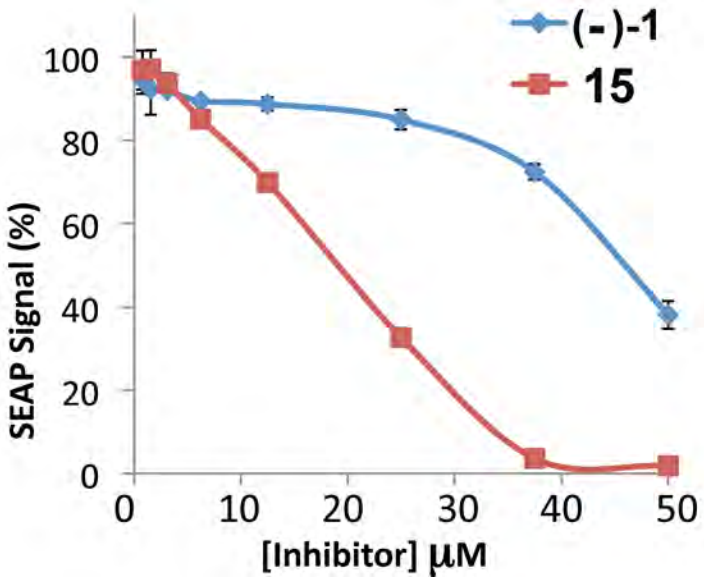
A mixture of appropriate *N,N′*-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediaminocobalt(II) (0.031 g, 0.051 mmol), and acetic acid (0.032 ml, 0.515 mmol) was stirred open to air at room temperature for 45 min. The solvent was removed by rotary evaporation. To the resulting residue was then added THF (2.06 ml), followed by racemic 2-((4-ethoxyphenol)methyl)oxirane (.200 g, 1.030 mmol), water (10.2 µl, 0.566 mmol). The reaction mixture was stirred for 16-20hrs. Upon completion, the solvent was removed and the

resulting residue was purified using silica gel column chromatography with 1:9 ethyl acetate:hexanes as an eluting solvent. The product was isolated as a white solid in a 92% (0.092 g) yield based on maximum theoretical yield.

Secreted alkaline phosphatase (SEAP) assay

Materials for the SEAP assay were obtained from Applied Biosystems (CA, USA) and utilized according to the manufacturer's specifications. Human embryonic kidney 293 (HEK293) cells stably transfected with TLR4 and a secreted alkaline phosphatase (SEAP) reporter gene was obtained from Invivogen (CA, USA). Cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 10× penicillin/streptomycin, 10× l-glutamine, 1× normocin (ant-nr-1) and 1× HEK Blue (hb-sel). Cells were implanted in 96 well plates for 24 h at 37 °C prior to drug treatment. On the day of treatment, media was removed from the 96-well plate, replaced with cerebrospinal fluid (CSF) buffer (124 mM NaCl, 5 mM KCl, 0.1 mM $CaCl_2$, 3.2 mM $MgCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose, pH 7.4) containing 10 ng/mL LPS, as well as 0.2-50.0 μM drug or 3-400 ng/mL LPS-RS (positive control) with 0.5% DMSO.

A sample of CSF buffer (15 μL) from each well was collected and transferred to an opaque white 96 well plate (Microfluor 2, Thermo Scientific MA, USA). Each well was treated with 45 μL of 1 × dilution buffer, covered with microseal (MSB1001, Bio-Rad, CA, USA) and incubated for 30 min at 65 °C. After 30 min, plates were cooled to room temperature on ice and 50 μL of SEAP assay buffer was added to each well. After a 5 min incubation, 50 μL of disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2-(5-chloro)tricyclo[3.3.1.13,7]decan}-4-yl) phenyl phosphate (CSPD) diluted 1:20 with reaction buffer was added to each well. After 20 min, the luminescence of each well was measured using a plate reader (Beckman Coulter, DTX 880, CA, USA) with multimode analysis software.



Supplementary Figure 1. Representative dose-dependent inhibitory effects by various concentrations of **(-)-1** and **15** inhibiting LPS (10 ng/ML) -induced TLR4 activation. The inhibition assay is validated by using a previously reported inhibitor, LPS-RS, as the positive control. Data are means from three independent experiments.

RAW264.7 nitric oxide cell TLR selectivity assay

RAW cells were grown in DMEM supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 mg/ml) and L-glutamine (2 mM). RAW cells were then planted in 96-well plates at 100,000 cells per well and grown for 24 h in the media descried previously. After 24 h media was removed and replaced with Macrophage-SFM (Invitrogen, CA, USA). Lanes were doped with the appropriate TLR specific ligands: LPS (lipopolysaccharide), poly(I:C) (polyinosinic-polycytidylic acid), FSL-1 ((*S*,*R*)-(2,3-bispalmitoyloxypropyl)-Cys-Gly-Asp-Pro-Lys-His-Pro-Lys-Ser-Phe), R848 (4-amino-2-(ethoxymethyl)-α,α-dimethyl-1*H*-imidazo[4,5-c]quinoline- 1-ethanol) and Pam_3CSK_4 (*N*-palmitoyl-*S*-[2,3-bis(palmitoyloxy)-

(2*RS*)-propyl]-[*R*]-cysteinyl-[*S*]-seryl-[*S*]-lysyl-[*S*]-lysyl-[*S*]-lysyl-[*S*]-lysine.3HCl) were used to selectively activate TLR4, TLR3, TLR2/6, TLR7 and TLR2/1 respectively. Two lanes for each ligand were prepared one containing ligand only and the other with the ligand and 300 nM 1. Plates were then incubated for 24 h. Following incubation 100 μL of media was removed and added to flat black 96-well microfluor plates (Thermo scientific, MA, USA). 10 μL of 2,3-diaminonaphthalene (0.05 mg/ml in 0.62 M HCl) was added to each well and incubated for 15 min. The reaction was quenched by addition of 5 μL 3 M NaOH and the plate was read on Beckman Coulter DTX880 reader (Beckman Coulter, CA, USA) with excitation at 365 nm and emission at 450 nm. Nitrite (a stable metabolite of nitric oxide) concentration was determined from a nitrite standard curve.

Computer-aided docking simulation.

The docking studies were performed using AutoDock 4.0. Lamarckian Generic Algorithm (LGA) and the torsion angles of the ligand were varied using AUTOTORS. All other procedures for the docking experiment were followed as described in the user manual for the AutoDock 4.0 program. Docked conformations were ranked automatically by the AutoDock 4.0 program using a force field scoring function. A total of 100 distinct conformational clusters were found out of 100 runs using an rmsd-tolerance of 1.0 Å. Among those, one of the highest three ranked docked structures was used for molecular visualization.

Cell viability assay

Human embryonic kidney 293 (HEK293) cells were stably transfected with TLR4 and necessary assembly and signalling proteins (MD2, CD-14, LPSBP, etc.). Cells were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 mg/ml), L-

glutamine (2 mM), 0.1 mg/ml normocin (InvivoGen, CA, USA) and 1 × HEK Blue selection reagent (InvivoGen, CA, USA). Cells were implanted in 6 cm plates and grown to 65-75% confluency by incubating at 37 °C, prior to drug treatment. On the day of treatment, media was removed from the 6 cm plate and replaced with cerebrospinal fluid (CSF) buffer supplemented with drug treatment. After 24 h incubation at 37 °C, CSF was removed and cells were agitated with 0.05% Trypsin plus 0.2 g/l EDTA (Invitrogen, CA, USA) and re-suspended in fresh DMEM supplemented media. After re-suspension, a 100 μl sample was taken from each 6 cm plate, mixed gently with 100 μl 0.4% Trypan Blue (Sigma, MO, USA) and allowed to sit for 5 min. The ratio of blue stained cells to total cells was then quantified using a Bright Line 0.1 mm depth hemocytometer (VWR, PA, USA) under a Nikon TMS light microscope (Nikon Instrumentals, CA USA).

Behavioral assessment of responsivity radiant heat in TLR4-knockout and wild-type mice

Pathogen-free adult male Sprague–Dawley rats (n=5-6 rats/group for each experiment; 300–375 gm; Harlan Labs, Madison, WI, USA) were used in all experiments. Rats were housed in temperature (23±3°C) and light (12 h:12 h light:dark cycle; lights on at 0700) controlled rooms with standard rodent chow and water available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder. All testing was conducted blind with respect to group assignment. Rats received at least three 60 min habituations to the appropriate test environment prior to behavioral testing. Morphine was gifted from Mallinkrodt (St. Louis, MO, USA). Thresholds for behavioral response to heat stimuli applied to the tail were assessed using a modified Hargreaves test. Briefly, baseline withdrawal values were calculated from an average of two consecutive withdrawal latencies of the tail, measured at 15 min intervals. Latencies for the thermal stimulus at baseline ranged from 2 to 3 s and a cut-off time of 10 s was imposed to avoid tissue damage. Baseline withdrawal latency assessments were performed prior to, and again across a timecourse after drug administration. Vehicles were administered equivolume to the drugs under test. Data is shown as the percentage maximum effect which is (test-baseline/10 s cut off -baseline)*100.