Supporting Information

The Discovery of MK-4409, a Novel Oxazole FAAH Inhibitor for the Treatment of Inflammatory and Neuropathic Pain

Harry R. Chobanian[a],* Yan Guo[a], Ping Liu[a], Marc D. Chioda [a], Selena Fung[a], Thomas J. Lanza[a], Linda Chang[a], Raman K. Bakshi[a], James P. Dellureficio[a], Qingmei Hong[a], Mark McLaughlin[b], Kevin M. Belyk[b], Shane W. Krska[b], Amanda K. Makarewicz[b], Elliot J. Martel[b], Joseph F. Leone[b], Lisa Frey[b], Bindhu Karanam[c], Maria Madeira[c], Raul Alvaro[c], Joyce Shuman[c], Gino Salituro[c], Jenna L. Terebetski[d], Nina Jochnowitz[e], Shruti Mistry[e], Erin McGowan[e], Richard Hajdu[e], Mark Rosenbach[e], Catherine Abbadie[e], Jessica P. Alexander[f], Lin-Lin Shiao[f], Kathleen M. Sullivan[f], Ravi P. Nargund[a], Matthew J. Wyvratt[a], Linus S. Lin[a], Robert J. DeVita[a]

[a] Departments of Medicinal Chemistry, [b] Process Chemistry, [c] Drug Metabolism and Pharmacokinetics, [d] Preclinical Development, [e]Pharmacology, [f]Immunology, Merck Research Laboratories, Kenilworth, NJ 07033, USA

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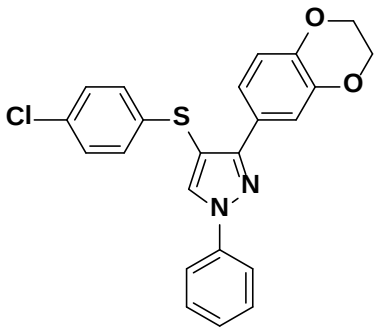
General Information. All reagents were purchased from Aldrich and used without further purification unless otherwise stated. Column chromatography was carried out on flash silica gel (Merck 230-400 mesh). TLC analysis was conducted on ANALTECH silica gel plates. The LC/MS analyses were performed using a MICROMASS ZMD mass spectrometer coupled to an AGILENT 1100 Series HPLC utilizing a YMC ODS-A 4.6 x 50 mm column eluting at 4.5 mL/min with a solvent gradient of 10 to 95% B over 2.5 min, followed by 0.5 min at 95% B: solvent A = 0.06% TFA in water; solvent B = 0.05% TFA in acetonitrile. ^{1}H-NMR spectra were obtained on a 500 MHz VARIAN Spectrometer in $CDCl_3$, CD_3OD, or Acetone-d_6 as indicated and chemical shifts are reported as δ using the solvent peak as reference and coupling constants are reported in hertz (Hz).

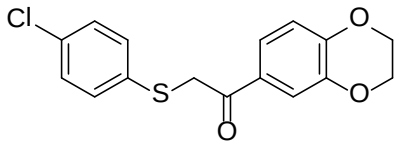
For Pan Labs screen, 168 radioligand binding or enzymatic assays were carried at MDS Pharma Services as a contract service to Merck. A summary of each assay protocol and the reference for each assay are listed in the MDS Pharma catalog.

All animal studies described herein were approved by the Merck Research Laboratories Institutional Animal Care and Use Committee.

4-((4-Chlorophenyl)thio)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-phenyl-1H-pyrazole (11)

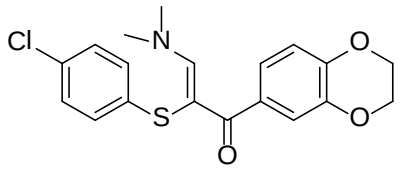


Step 1: 2-((4-Chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone (18a)



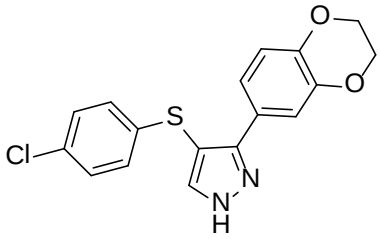
To a stirred solution of 4-chlorobenzenethiol (816 mg, 5.6 mmol) in THF (30 mL) was added DIPEA (1.2 mL, 7 mmol) at 0°C. At which point, a solution of 2-chloro-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone (1 g, 4.7 mmol) in THF (35 mL) was added dropwise. The resulting solution was allowed to warm to rt and stir for 96 h. The solution was diluted with water (50 mL) and EtOAc (150 ml). The organic layer was removed, dried, filtered and concentrated giving rise to an oil. The residue was purified by flash chromatography (0-40% EtOAc in hexanes) to afford 1.1 g (yield 77%) of 2-((4-chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone. LCMS: m/z 321 $(M+H)^+$.

Step 2: 2-((4-Chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(dimethylamino)prop-2-en-1-one



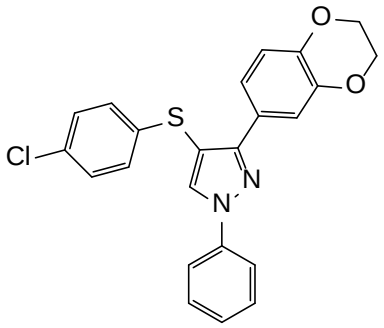
A stirred solution of 2-((4-chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone (1.6 g, 5 mmol) was dissolved in DMF-DMA (5 mL, 35 mmol). The resulting solution was heated to 100°C for 40 minutes. Volatiles were removed under reduced pressure and the residue was taken up in ethyl acetate (100 mL) and water (50 mL), and the phases were separated. The organic phase was washed with water, brine, and dried (anhyd. $MgSO_4$). The residue was purified by flash chromatography (0-100% EtOAc in hexanes) to afford 1.1 g (yield 59%) of 2-((4-chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(dimethylamino)prop-2-en-1-one. LC/MS: *m/e* 345.0 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 4.29 (4H, d, *J* = 3.7 Hz), 6.90 (1H, d, *J* = 8.2 Hz), 7.03 (2H, d, *J* = 8.2 Hz), 7.20 (1H, d, *J* = 8.2 Hz), 7.25 (1H, d, *J* = 8.4 Hz), 7.30 (2H, d, *J* = 9.1 Hz), 7.78 (1H, s).

Step 3: 4-((4-Chlorophenyl)thio)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1H-pyrazole (19a)



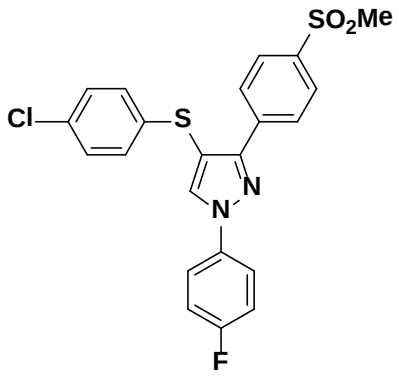
A stirred solution of 2-((4-chlorophenyl)thio)-1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-3-(dimethylamino)prop-2-en-1-one (540 mg, 1.4 mmol) in EtOH (3 mL) was treated with hydrazine monohydrate (0.14 mL mmol, 2.9 mmol) and the mixture was heated at 75° C for 30 minutes. Upon completion of the reaction as judged by LC/MS, the solution was concentrated to dryness and the residue was purified by flash chromatography (0-100% EtOAc in hexanes) to afford 440 mg (yield 89%) of 4-((4-chlorophenyl)thio)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1H-pyrazole. LC/MS: *m/e* 345.0 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 4.29 (4H, d, *J* = 3.7 Hz), 6.90 (1H, d, *J* = 8.2 Hz), 7.03 (2H, d, *J* = 8.2 Hz), 7.20 (1H, d, *J* = 8.2 Hz), 7.25 (1H, d, *J* = 8.4 Hz), 7.30 (2H, d, *J* = 9.1 Hz), 7.78 (1H, s).

Step 4: **4-((4-Chlorophenyl)thio)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1-phenyl-1H-pyrazole (11)**



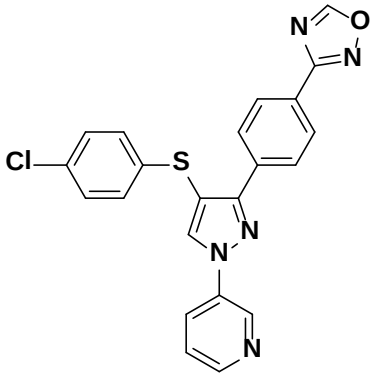
A solution of 4-((4-chlorophenyl)thio)-3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-1H-pyrazole (35 mg, 0.1mmol), iodobenzene (172 mg, 0.8 mmol), *trans-N,N'*-dimethyl-1,2-cyclohexanediamine (64 mg, 0.4 mmol), CuI (87 mg, 0.4 mmol), K_3PO_4 (70 mg, 0.5 mmol) in Acetonitrile (3 mL) was heated to 120 ^oC in a sealed tube. Upon completion of the reaction as judged by LC/MS, the solution was concentrated to dryness and purified by flash chromatography (0-100% EtOAc in hexanes) to afford 33 mg (yield 79%) of **11**. LC/MS: *m/e* 421.2 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 4.29 (4H, s), 6.88 (2H, d, *J* = 8.5 Hz), 7.10 (2H, d, *J* = 8.7 Hz), 7.20 (1H, d, *J* = 8.5 Hz), 7.35 -7.38 (3H, m), 7.50-7.55 (3H, m), 7.60 (2H, d, *J* = 7.8 Hz), 8.16 (1H, s).

4-((4-Chlorophenyl)thio)-1-(4-fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-1H-pyrazole (12)



The target compound was prepared from 4-((4-chlorophenyl)thio)-3-(4-(methylsulfonyl)phenyl)-1H-pyrazole analogously to compound **11** except that 1-fluoro-4-iodobenzene was used instead of iodobenzene. LC/MS: *m/e* 458.9 $(M+H)^+$. ^1H NMR (500 MHz, $CDCl_3$): δ 3.08 (3H, s), 7.09 (2H, d, *J* = 8.5 Hz), 7.22-7.25 (4H, m), 7.78-7.80 (2H, m), 7.96 (2H, d, *J* = 8.5 Hz), 8.17 (1H, s), 8.26 (2H, d, *J* = 8.5 Hz).

3-(4-(4-((4-Chlorophenyl)thio)-1-(pyridin-3-yl)-1H-pyrazol-3-yl)phenyl)-1,2,4-oxadiazole (13)



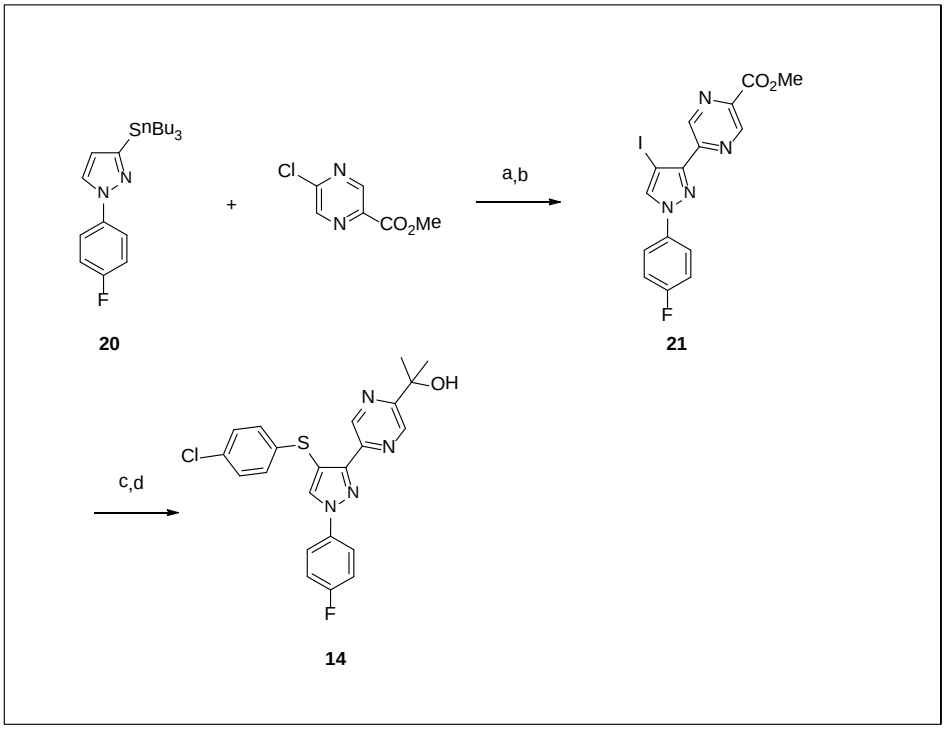
Step 1: 4-(4-((4-chlorophenyl)thio)-1H-pyrazol-3-yl)benzonitrile (**19c**) (prepared as previously described for example **19a** using 4-(2-bromoacetyl)benzonitrile and 4-chlorobenzenethiol)(1.2 g, 4 mmol), 4-iodopyridine (0.9 g, 4.4 mmol), CuI (0.08 g, 0.4 mmol), K_2CO_3 (1.1 g, 8 mmol), DL-Proline (0.09 g, 0.8 mmol) were combined in DMSO (8 mL) and heated to 80°C for 12 h. After which point, the reaction was cooled to rt, diluted with EtOAc (150 mL) and washed with brine (100 mL). The

organic layer was removed, dried, filtered and concentrated giving rise to a residue which was purified by flash chromatography (0-100% EtOAc in hexanes) to afford 1.5 g (yield 96%) of 4-(4-((4-chlorophenyl)thio)-1-(pyridin-3-yl)-1H-pyrazol-3-yl)benzonitrile. LC/MS: *m/e* 389.1 $(M+H)^+$.

Step 2: 4-(4-((4-chlorophenyl)thio)-1-(pyridin-3-yl)-1H-pyrazol-3-yl)benzonitrile (100 mg, 0.3 mmol) was dissolved in EtOH and treated with hydroxylamine (0.05 mL, 0.7 mmol, 50% aqueous). The resulting solution was heated to 80°C for 40 mins. After which point, the solution was concentrated to dryness to afford 4-(4-((4-chlorophenyl)thio)-1-(pyridin-3-yl)-1H-pyrazol-3-yl)-*N*-hydroxybenzimidamide which was used crude in the next step.

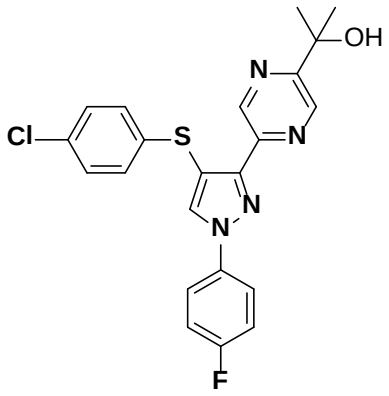
Step 3: 4-(4-((4-chlorophenyl)thio)-1-(pyridin-3-yl)-1H-pyrazol-3-yl)-*N*-hydroxybenzimidamide (162 mg, 0.4 mmol) was dissolved in triethylorthoformate (3 mL) and treated with TsOH (7 mg, 0.04 mmol). The resulting solution was heated to 80°C for 10 h. After which point, the solution was concentrated and purified by flash chromatography (0-100% EtOAc in hexanes) to afford 156 mg (yield 94%) of **13**. LC/MS: *m/e* 432.1 $(M+H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.04 (2H, d, *J* = 5 Hz); 7.12 (2H, d, *J* = 5 Hz); 7.43 (1H, dd, *J* = 8.3, 4.8 Hz); 8.15-8.06 (6H, m); 8.56 (1H, d, *J* = 4.9 Hz); 8.69 (1H, s); 9.02 (1H, s).

Synthetic Scheme for analogue **14:**

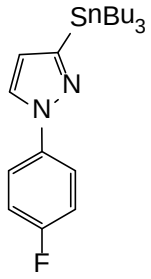


(a) $Pd(PPh_3)_4$, LiCl, THF, 70°C, 73%; (b) NIS, TFA, CH_3CN, 63%; (c) 4-ClPhSH, NaH, CuI, NMP, 150°C, 82%; (d) MeMgBr, THF, rt, 43%.

2-(5-(4-((4-Chlorophenyl)thio)-1-(4-fluorophenyl)-1H-pyrazol-3-yl)pyrazin-2-yl)propan-2-ol (14)

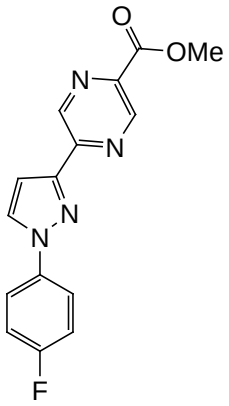


Step 1: 1-(4-Fluorophenyl)-3-(tributylstannyl)-1*H*-pyrazole (20)



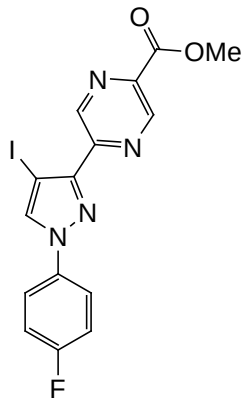
The title compound was prepared using the procedure described by Sakamoto, T.; Shiga, F.; Uchiyama, D.; Kondo, Y.; Yamanaka, H. *Heterocycles* **1992**, *33*, 813 or Chobanian, H.R.; Lin, L.S.; Liu, P.; Chioda, M.D.; Devita, R.J.; Nargund, R.P.; Guo, Y.; Preparation of oxazole derivatives useful as inhibitors of FAAH. *Patent Application US2011/0021531-A1*, 2011.

Step 2: **Methyl 5-[1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinecarboxylate**



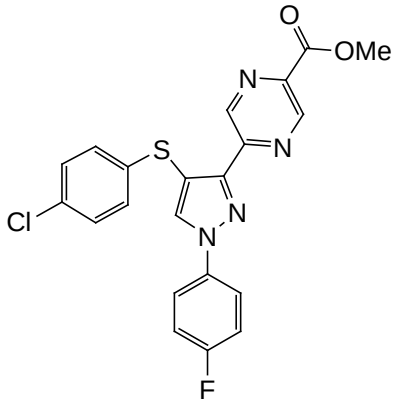
A solution of 1-(4-fluorophenyl)-3-(tributylstannyl)-1*H*-pyrazole (1.4 g, 3.2 mmol), methyl 5-chloropyrazine-2-carboxylate (500 mg, 2.9 mmol), $Pd(PPh_3)_4$ (670 mg, 0.60 mmol) and LiCl (368 mg, 8.7 mmol) in THF (20 mL) was heated to 70° C for 12h. Upon completion of the reaction as judged by TLC analysis, the solution was diluted with distilled H_2O and extracted with EtOAc. The organic layer was removed, dried over $MgSO_4$, filtered and concentrated giving rise to an oil. The oil was purified on silica gel to give rise to 630 mg (73%) of title compound. LC/MS: *m/e* 299.1 $(M+H)^+$. ^1H NMR (500 MHz, Acetone-d_6): δ 3.99 (s, 3H), 7.25 (d, *J* = 2.5 Hz, 1H), 7.34-7.49 (m, 2H), 8.02 (m, 2H), 8.52 (d, *J* = 2.5 Hz, 1H), 9.21 (d, *J* = 1.0 Hz, 1H), 9.43 (d, *J* = 1.5 Hz, 1H).

Step 3: Methyl-5-[1-(4-fluorophenyl)-4-iodo-1H-pyrazol-3-yl]-2-pyrazinecarboxylate (21)



A solution of methyl 5-[1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinecarboxylate (200 mg, 0.70 mmol), NIS (181 mg, 0.80 mmol), TFA (1 mL) in CH_3CN (30 mL) was stirred at rt for 1 h. Upon completion of the reaction as judged by TLC analysis, the solution was diluted with sat aq $NaHCO_3$ and extracted with EtOAc. The organic layer was removed, dried over $MgSO_4$, filtered and concentrated giving rise to an oil. The oil was purified on silica gel to give rise to 178 mg (63%) of compound **21**. LC/MS: *m/e* 425.0 $(M+H)^+$. ^1H NMR (500 MHz, Acetone-d6): δ 4.01 (s, 3H), 7.37-7.41 (m, 2H), 8.04 (m, 2H), 8.70 (s, 1H), 9.28 (d, *J* = 1.5 Hz, 1H), 9.44 (d, *J* = 1.0 Hz, 1H).

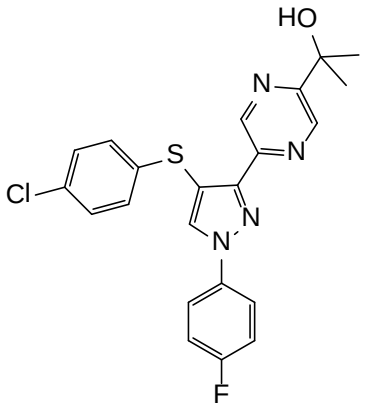
Step 4: Methyl-5-[4-[(4-chlorophenyl)thio]-1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinecarboxylate



A solution of 4-chlorothiophenol (64 mg, 0.4 mmol) in 10 mL of NMP was treated with NaH (17 mg, 0.4 mmol). The solution was stirred for 15 minutes at rt. After which point, compound **21** (170 mg, 0.4

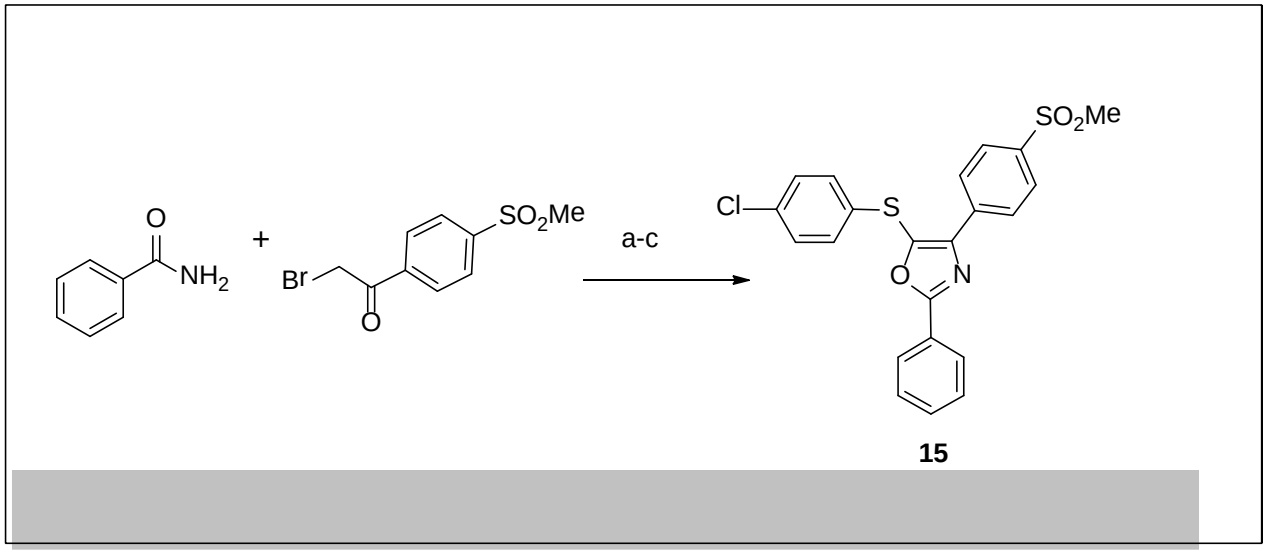
mmol) was added followed by CuI (76 mg, 0.4 mmol). The resulting solution was heated to 150°C. Upon completion of the reaction as judged by TLC analysis, the solution was diluted with sat H_2O (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were removed, dried over $MgSO_4$, filtered and concentrated giving rise to an oil. The oil was purified on silica gel to give rise to 145 mg (82%) of methyl-5-[4-[(4-chlorophenyl)thio]-1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinecarboxylate. LC/MS: *m/e* 441.0 $(M+H)^+$.

Step 5: 2-{5-[4-[(4-Chlorophenyl)thio]-1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinyl}-2-propanol (14)



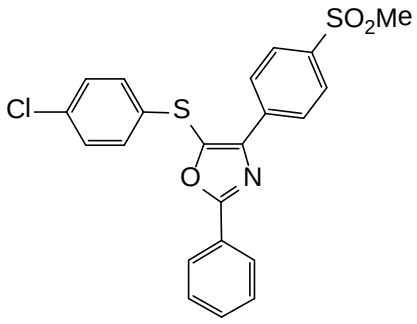
Step 3: Methyl-5-[4-[(4-chlorophenyl)thio]-1-(4-fluorophenyl)-1*H*-pyrazol-3-yl]-2-pyrazinecarboxylate (70 mg, 0.2 mmol) was dissolved in THF (5 mL). At which point, MeMgBr (0.5 mL, 1.6 mmol, 3.0 M in THF) was added dropwise at rt. The resulting solution was stirred for 20 min at rt. After which point, the solution was quenched with sat NH_4Cl solution. The solution was extracted with EtOAc (100 ml). The organic layer was removed, dried over $MgSO_4$, filtered and concentrated to give rise to an oil. The oil was purified by flash chromatography (4-50% EtOAc in hexanes) to afford 30 mg (yield 43%) of **14**. LC/MS: *m/e* 441.1 $(M+H)^+$. ^1H NMR (500 MHz, Acetone-d_6): δ ppm 1.58 (6H, s); 7.32-7.38 (6 H, m); 8.03-8.06 (2H, dd, *J* = 4.5, 9.5 Hz); 8.57 (1H, s); 8.95 (1H, s); 9.13 (1H, s).

Synthetic Scheme for Analogue **15:**

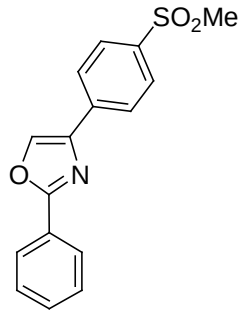


(a) neat, 150°C, 30%; (b) Br_2, HOAc, $CHCl_3$, rt, 80%; (c) 4-ClPhSH, KOH, EtOH, 80°C, 80%.

5-[(4-Chlorophenyl)thio]-4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole (15)

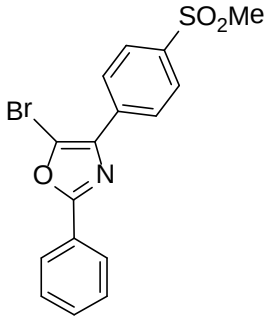


Step 1: 4-[4-(Methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole



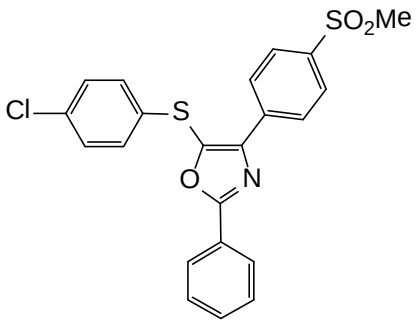
A mixture of 2-bromo-1-[4-(methylsulfonyl)phenyl]ethanone (2 g, 7.2 mmol) and benzamide (0.87 g, 7.2 mmol) was heated to 150℃ for 4 h. When TLC showed that the reaction had completed, the mixture was cooled, and partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc (2 x 20 mL), and the combined organic layers were washed with water, brine, dried over $MgSO_4$, and filtered. After concentration, the residue was purified by column (eluted by PE:EA=10:1) to afford 0.6 g (yield 30%) of 4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole.

Step 2: 5-Bromo-4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole



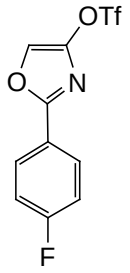
To a solution of 4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole (0.7 g, 2.3 mmol) in AcOH (20 mL) and $CHCl_3$ (30 mL) was added dropwise Br_2 (0.41 g) at rt. The resulting mixture was stirred for 2 h at rt. The reaction mixture was poured into water, and extracted with EtOAc (3 x25 ml). The combined organic layers were washed with aqueous $NaHCO_3$, brine, and dried over Na_2SO_4. After concentration, the residue was purified by column (PE:EA = 4:1) to afford 0.7 g (yield 80%) of 5-bromo-4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole. LC/MS: *m/e* 396.0$(M+H)^+$.

Step 3: 5-[(4-Chlorophenyl)thio]-4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole (15)



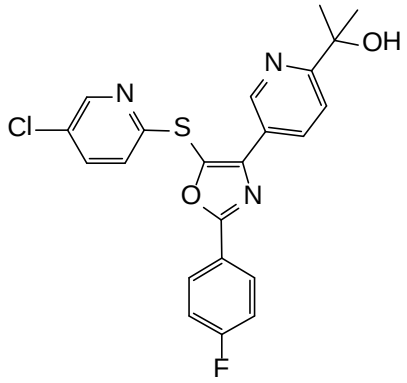
To a solution of 5-bromo-4-[4-(methylsulfonyl)phenyl]-2-phenyl-1,3-oxazole (0.2 g, 0.5 mmol) and 4-chlorbenzenethiol (0.08 g, 0.5 mmol) in EtOH (10 ml) was added KOH (34 mg, 0.6 mmol) at rt under N_2, then the mixture was heated to reflux overnight. After cooling, the precipitate was collected by suction, and the filter cake was washed with EtOH. After drying, 200 mg (yield 80%) of the compound **15** was obtained. LC/MS: *m/e* 442.1$(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO) δ 8.30 (d, 2 H, Ar-H), 8.06 (m, 4 H, Ar-H), 7.60 (m, 3 H, Ar-H), 7.40 (m, 4H, Ar-H), 3.26 (s, 3 H, CH_3).

2-(4-Fluorophenyl)-1,3-oxazol-4-yl trifluoromethanesulfonate (22)

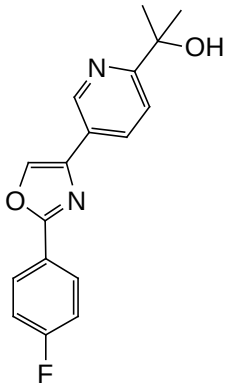


The title compound was prepared using the procedure described by Langille, N.F.; Dakin, L.A.; Panek, J.S. *Org. Lett.* **2002**, *4*, 2485.

2-(5-(5-((5-Chloropyridin-2-yl)thio)-2-(4-fluorophenyl)oxazol-4-yl)pyridin-2-yl)propan-2-ol (17)

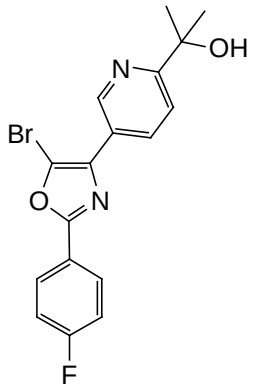


Step 1: 2-{5-[2-(4-Fluorophenyl)-1,3-oxazol-4-yl]pyridin-2-yl}propan-2-ol (23 b)



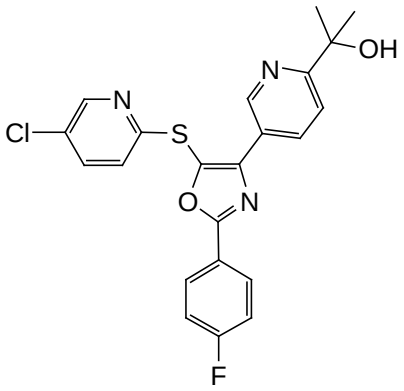
A solution of 2-(4-fluorophenyl)-1,3-oxazol-4-yl trifluoromethanesulfonate (60 g, 0.20 mol), bis-pinacolatodiboron (500g, 0.25 mol), KOAc (57.0, 0.58 mol), $Pd(dppf)Cl_2$ (7.90 g, 9.60 mmol), and dppf (5.34g, 9.60 mmol) in 1,4-dioxane (1.6 L) were heated to 101°C for 3 h. Upon completion of the reaction as judged by TLC analysis, the reaction was allowed to cool to 65°C. At which point, 2-(5-bromopyridin-2-yl)propan-2-ol (62.6 g, 0.30 mol) and $Pd(PPh_3)_2Cl_2$ (13.6 g, 0.02 mol) were added followed by dropwise addition of aqueous Na_2CO_3 (193 mL, 0.40 mol, 2 M). The solution was heated to 91°C for 12 h. Upon completion of the reaction as judged by LC/MS analysis, the solution was diluted with dist H_2O and extracted with EtOAc (2x). The combined organic layers were removed, dried over $MgSO_4$, filtered and concentrated giving rise to an oil. The oil was purified on silica gel to give afford the title compound (38.50 g, 67%). LC/MS: *m/e* 299.1 (M+H).

Step 2: 2-{5-[5-Bromo-2-(4-fluorophenyl)-1,3-oxazol-4-yl]pyridin-2-yl}propan-2-ol



A solution of 2-{5-[2-(4-Fluorophenyl)-1,3-oxazol-4-yl]pyridin-2-yl}propan-2-ol (38.5 g, 0.13 mol) and NBS (28.0 g, 0.16 mol) in CH_2Cl_2 (1.3 L) was stirred at rt for 12 h. Upon completion of the reaction, the solution was diluted with sat aq NaS_2O_3 solution. The organic layer was removed, dried over $MgSO_4$, filtered and concentrated giving rise to an oil. The oil was purified on silica gel to afford the title compound (31.97 g, 66%). LC/MS: *m/e* 377.0 $(M+H)^+$.

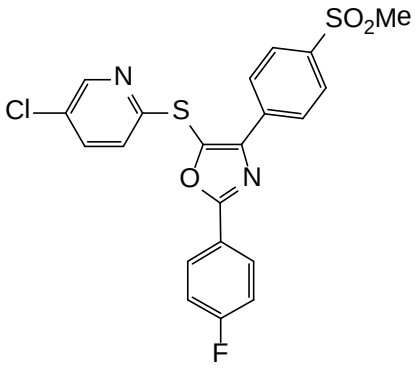
Step 3: 2-{5-[5-[(5-Chloropyridin-2-yl)thio]-2-(4-fluorophenyl)-1,3-oxazol-4-yl]pyridin-2-yl}propan-2-ol (17)



A solution of 5-chloropyridine-2-thiol (27.3 g, 0.20 mol) dissolved in 200 mL of NMP was treated with NaH (7.7 g, 0.20 mol). The resulting solution was stirred for 30 min at rt before 2-{5-[5-Bromo-2-(4-fluorophenyl)-1,3-oxazol-4-yl]pyridin-2-yl}propan-2-ol (31.9 g, 0.08 mol) dissolved in 200 mL of NMP was added by addition funnel. Lastly, CuI (16.3 g, 0.08 mol) was added to the solution. The resulting dark solution was heated to 120°C for 2 h. After which point, the solution was cooled to rt. Once at rt, the solution poured into a rapidly stirred solution of 9:1 NH_4Cl:NH_4OH and EtOAc. Upon clarification, the organic layer was removed followed by drying over $MgSO_4$, filtration and concentration giving rise to an oil. The oil was purified on silica gel to

afford the title compound (31.87 g, 85%). LC/MS: *m/e* 442.1 $(M+H)^+$. ^{1}H NMR (500 MHz, Acetone-d_6): δ 1.76(s, 6H), 5.01 (s, 1H), 7.40(m, 3H), 7.80 (m, 2H), 8.25 (m, 2H), 8.44 (dd, *J* = 2.3, 8.2 Hz, 1H), 8.44 (d, *J* = 2.3 Hz, 1H), 9.20 (d, *J* = 1.4 Hz, 1H).

5-((5-chloropyridin-2-yl)thio)-4-(4-(methylsulfonyl)phenyl)-2-phenyloxazole (16)



Compound **16**: LC/MS: *m/e* 460.7 $(M+H)^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.09 (s, 3H), 7.05 (d, *J* = 8.5 Hz, 1H), 7.22(m, 2H), 7.56 (m, 1H), 8.01 (d, *J* = 8.5 Hz, 2H), 8.19 (m, 2H), 8.37 (d, *J* = 8.5 Hz, 2H), 8.41 (d, *J* = 2.5 Hz, 1H).

Table 4. Pharmacokinetics of Compound 17 (MK-4409) across Rat, Dog and Monkey

Species	Cl (mL/min•kg)	Vd (L/kg)	$T_{1/2}$ (h)	F(%)[d]
Rat	20	6.2	4.3	120
Dog	1.4	3.2	6.1	62
Monkey	3.7	2.1	6.8	120

Pharmacokinetics were determined at doses of 1 mg/kg i.v. and 2 mg/kg p.o. in rats, and at 0.5 mg/kg i.v. and 1 mg/kg p.o. in dogs and monkeys. Oral pharmacokinetics were determined using a solution formulation in Ethanol:PEG400:water (20:40:40, *v/v/v*) for **17.**

FAAH assays. Enzyme activity was demonstrated in a radioenzymatic test based on measuring the product of hydrolysis (ethanolamine [^{3}H]) of anandamide [ethanolamine 1-.sup.3H] (American Radiolabeled Chemicals; 1mCi/ml) with FAAH (Life Sciences (1995), 56, 1999-2005 and Journal of Pharmacology and Experimented Therapeutics (1997), 283, 729-734), Analytical. Biochemistry (2003), 318, 270-5. In addition, routine assays were performed monitoring hydrolysis of arachidonyl-7-amino-4-methylcoumarin amide (AAMCA) by following increase in fluorescence upon release of 7-amino 4-methyl coumarin (λ_{EX}= 355 nm, (λ_{EM} =460 nm). Analytical. Biochemistry (2005). 343, 143-51.

Assays are performed on either cell lysate or microsome fractions employing either the fluorescent substrate AAMCA (Cayman chemical, Ann Arbor, MI,) or ^{3}H-anandamide ([ETHANOLAMINE- 1-3H] American Radiolabeled Chemicals; 1mCi/mL). The cell lysate or microsome assay is performed in Costar black wall, clear bottom plates by adding FAAH_CHO (whole cell, cell lysate or microsome) in assay buffer (50 mM Phosphate, pH 8.0, 1 mM EDTA, 200 mM KCl, 0.2% glycerol, 0.1% fatty acid free BSA) to each well, followed by either DMSO or compound and allowed to incubate at 22-25^{0}C for fifteen minutes. AAMCA substrate was used to achieve a final concentration of 1 μM and reaction allowed to proceed at room temperature for 1-3 hours. Fluorescent release as a measure of FAAH activity was monitored by reading the plate in a CytoFluor Multiplate Reader (Ex: 360/40nM; Em: 460/40nM).

CFA Study Methods

The Guide and Animal Welfare regulations were followed in the conduct of the animal studies. Veterinary care was given to any animals requiring medical attention.

Animals: Male SD rats from Charles River are housed 2 per cage with a 12 hour light/dark cycle (7pm lights off). The rats were shipped at 9-10 weeks of age (350-375 gram) and were fed standard rat chow and water ad lib. Animals are housed for one week before study is initiated.

Formulation of CFA for injection: Freund's Adjuvant Complete, Sigma-Aldrich F5881. CFA is dilute 50% with 0.9% sterile Saline, and vortexed vigorously to emulsify CFA in shipment. Pour that saline into 22 ml vial with majority of CFA. . It is stored at 4^{o}C refrigerator preparation with saline and is used up till 30 days post mixing.

CFA dosing in the hindpaw: Three days prior to the CFA study; prepare 1 ml syringes (BD sterile syringe part # 309602), containing 600ul of formulated CFA, with a 25G5/8" needle (BD). The rat is restrained and iinject interplanter (the ventral side) of the left foot toward the heel, 200ul CFA solution. The animal is returned to housing for three days or until the study commensed.

Testing phase: For the start of the study, rats were fasted twelve hours before the first drug dose, thereafter no fasting was done. All drugs are given PO (orally) and at a volume of 2 ml/kg.

Animals are tested for pain thresholds using a modified Randall-Sellito Paw pressure instrument. Initial baselining is done to the ipsi and contra-lateral paws before a test compound is administered. The paw pressure test applied increasing force to the paw. The test is stopped when either the subject responds by pulling the foot out of the test frame or if animal vocalizes. Baseline interval testing was in one hour intervals. After the second baseline test, the animals were dosed and all animals were tested thereafter on the ipsi-lateral paw. The contra-lateral paw BL is used for analysis.

On day one, animals were baseline tested, tested 1 and 3 hour post drug administration. In chronic phase of the CFA study, CFA was tested on only Day 1, 3, 5, 8 and 12 to decrease the potential in hypersenstiviety in the Paw pressure model. Also after day one testing, animals were split into two groups (day 3, 5, 8, 12), either they bofore drug administraion or after, not both times. Post drug administration paw pressure test was at 3 hours. All CFA studies were blinded to the investigator.

SNL Study Methods

Animals: The rats were shipped from Harlan at 3 weeks of age (100-115 gram) and were fed standard rat chow and water ad lib. Animals were housed for one week before surgery is completed. Male SD rats were housed 2 per cage with a 12 hour light/dark cycle (7pm lights off).

Surgery: Modified SNL: Under an inhalational anesthetic, isoflurane, the left L5 spinal nerve is exposed and ligated with 6-0 silk, (mSNL model). The muscle overlying the area was closed with a continuous running stitch (4-0 suture) and the wound approximated with surgical steel staples. External staples were removed ten days after surgery.

Static Allodynia using Von Frey Filaments - Testing of animals: Animals are conditioned in individual polycarbonate boxes on a raised platform, and allowed to acclimate for ~2 hours the day before and on the day of the experiment for one hour. Withdrawal threshold values are assessed on 5 animals at a time. Von Frey filaments were applied to each paw in an ascending fashion for approximately 4 seconds, starting with the #5 filament until a positive response or a cut-off value #8 filament is reached. The cutoff value (#8) was recorded even if there is no withdrawal response at this force. A positive response was recorded as “X” and a negative response as “0”. Each filament is applied @ 1 cm proximal to the edge of the heel, and to the lateral 1/3 of the hindpaw (the area innervated by the intact sural nerve). Testing is initiated on the ipsilateral paw (injured paw). A total of 6 values, after the first initial positive response, were recorded and used to calculate the 50% withdrawal threshold. The animals are not tested twice before surgery, and the study is run on day 28 post surgery. The values represented graphically.

Mechanical thresholds ipsi-lateral to the injury is determined prior to dosing & post dose @ 1 & 3 hours and 28 days after nerve injury surgery. Changes in mechanical thresholds were analyzed by a non-parametric ANOVA (Friedman) or t-test (Wilcoxon), where appropriate. Percent maximal possible effect (%MPE) was calculated as: (post-treatment – pre-treatment) / (pre-injury threshold – pre-treatment) X 100. For acute PO dosing studies, test animals were fasted 24 hours prior to initial dose and were tested at 1 & 3 hr post dose.