Supplementary Material

The discovery of AZD-2098 and AZD-1678, two potent and bioavailable CCR4 receptor antagonists.

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Experimental section:

All reagents and solvents were obtained from commercial suppliers and used without further purification. All reactions were carried out under an inert atmosphere of nitrogen unless otherwise noted. Silica gel chromatography was performed using either glass columns packed with silica gel (200–400 mesh, Aldrich Chemical) or prepacked silica gel cartridges (Biotage and ISCO). ^{1}H and ^{13}C NMR spectra were obtained from either a 300 MHz (^{1}H: 300 MHz, ^{13}C: 75 MHz) or 400 MHz (^{1}H: 400 MHz, ^{13}C: 100 MHz) Varian Unity Inova spectrometer. ^{1}H and ^{13}C shifts are given in ppm., δ scale and are measured relative to tetramethylsilane as standard. All final compounds were purified to >95% purity as determined by LCMS analysis obtained on Agilent 1100; Low-resolution mass spectral (MS) data were determined on a Hewlett Packard HP 6890 or HP5973 MSD mass spectrometer using ES ionization modes (positive or negative).

The synthetic procedures for the compounds listed within the communication are described in detail in either WO2003059893 or WO2007069978. However, to assist in the reproducibility of the synthesis the authors include the small scale synthetic procedures and analytical data for AZD2098 and AZD1678.

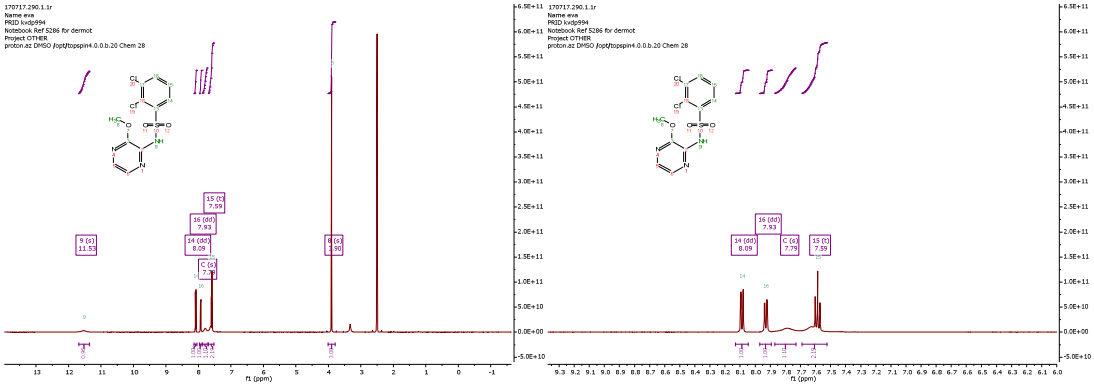
GENERAL/TYPICAL PROCEDURE:

2,3-Dichloro-N-(3-methoxypyrazin-2-yl)benzenesulfonamide (**47**)

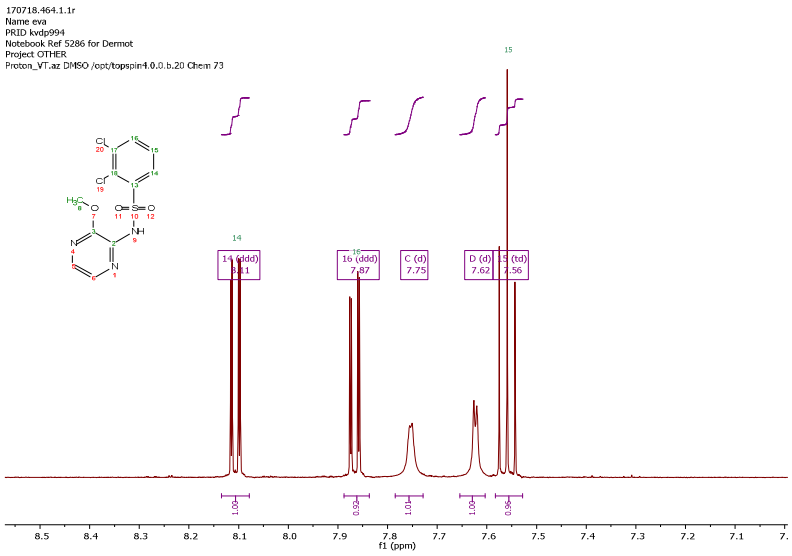
Potassium tert-butoxide (4.4 mL of 1M solution in tetrahydrofuran) was added dropwise to a stirred solution of 3-methoxy-2-pyrazinamine (0.25 g, 2 mmol)) and 2,3-dichlorobenzenesulfonyl chloride (0.515 g, 2.1 mmol) in dry tetrahydrofuran (10 mL) cooled in an ice bath. After 0.5 h, the reaction mixture was quenched with 2M aqueous hydrochloric acid (50 mL). The mixture was extracted with ethyl acetate (2 x 25 mL). The ethyl acetate layer was dried ($MgSO_4$) and evaporated. Purification was by silica gel chromatography eluting with ethyl acetate *iso*-hexanes 1:3. The solvent was evaporated to afford the product (0.59 g, 89%).
m.p. 187-188°C

LCMS (100% purity): m/e 334 $(M+H)^+$
The NMR contained 2 broad peaks for the C-5 and C-6 protons in DMSO. The 13C signals were not resolved therefore extensive work was undertaken and the optimal solvent to resolve the peaks was CD_3CN

^{1}H NMR (500 MHz, DMSO, 300K) 3.90 (s, 3H), 7.59 (t, J = 8.0 Hz, 1H), 7.62 (s, 1H, BROAD), 7.79 (s, 1H, BROAD), 7.93 (dd, J = 1.5, 8.1 Hz, 1H), 8.09 (dd, J = 1.5, 8.0 Hz, 1H), 11.53 (s, 1H).



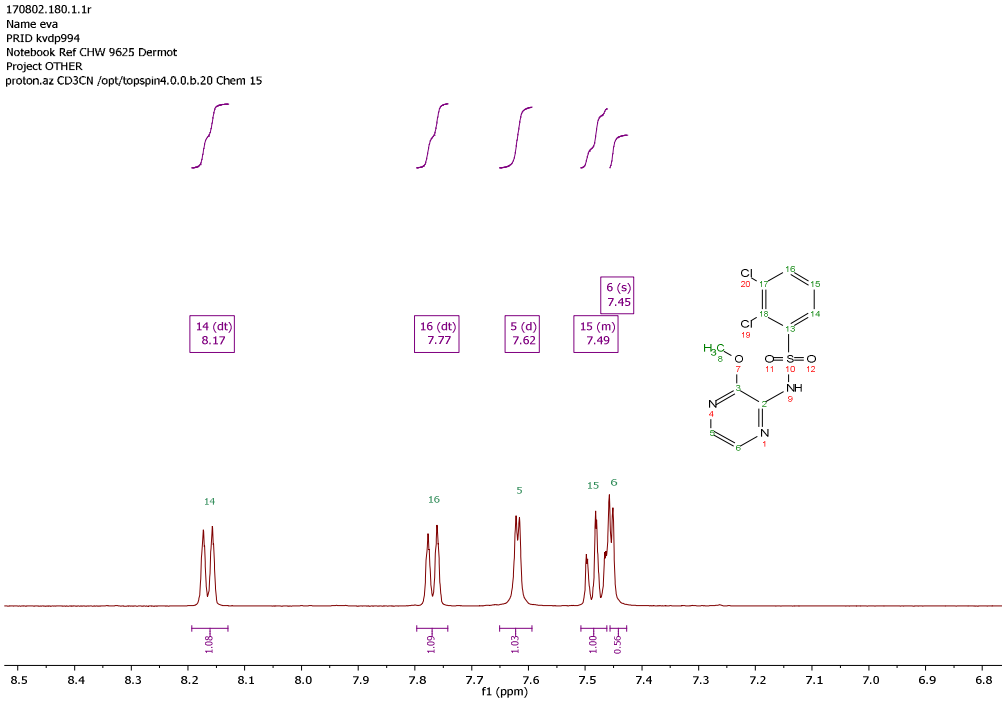
Sample heated to 373K



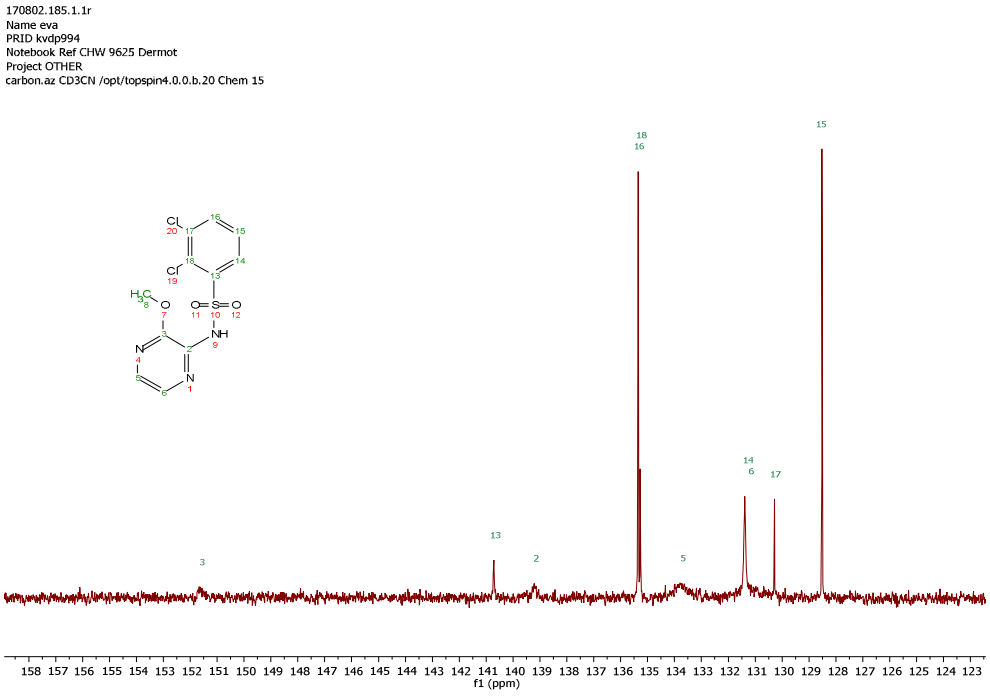
^{1}H NMR for compound **37** in DMSO

^{1}H NMR (500 MHz, CD_3CN, 300K) 3.94 (s, 3H), 7.45 (d, 1H), 7.49 (m, 1H), 7.62 (d, J = 3.2 Hz, 1H), 7.77 (dt, J = 1.5, 8.1 Hz, 1H), 8.17 (dt, J = 1.4, 8.1 Hz, 1H). (CD_3CN, referenced to 1.94 ppm)

^{13}C NMR (126 MHz, CD_3CN, 300K) 54.36, 128.58, 130.28 (broad), 131.18, 131.39, 133.73, 135.27 (broad), 135.35, 139.16, 140.69, 151.59. (CD_3CN, referenced to 118.07 ppm)

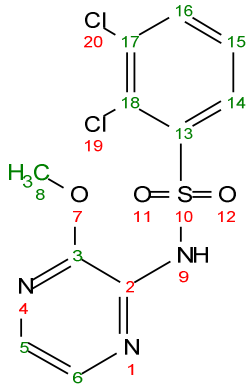


^{1}H NMR for compound **37** in CDCN



^{13}C NMR for compound **37** in CDCN

In CD_3CN (300K):



No	^{1}H-Chemical Shift (ppm)	Multiplicity (*J* value)	^{13}C- Chemical Shift (ppm)
14	8.17	dt,*J*=8.07,1.41 Hz	131.39
16	7.77	dt,*J*=8.12,1.45 Hz	135.35
15	7.49	m	128.58
8	3.94	s	54.36
5	7.62	d, *J* = 3.2 Hz	133.73 (broad)
6	7.45	d, *J* = 3.2 Hz	131.18 (broad)
2			139.16
3			151.59
13			140.69
17			135.27
18			130.28

2,3-Dichloro-*N*-(3-methoxy-5-nitropyrazin-2-yl)benzenesulfonamide (**52**)

Fuming nitric acid (1.26 g) was added dropwise to a stirred suspension of 2,3-dichloro-N-(3-methoxypyrazin-2-yl)benzenesulfonamide **47** (4.5 g, 13.5 mmol) in acetic acid (45 mL) at room temperature. The reaction was carefully heated to 75°C. After
l h. the reaction mixture was allowed to cool and the white crystalline product collected by filtration (3.94 g, 77 %).

^{1}H NMR (D6-DMSO) δ 8.53 (111, s), 8.16 (111, d), 7.95 (1H, d), 7.61 (1H, t) 4.02 (3H, s).

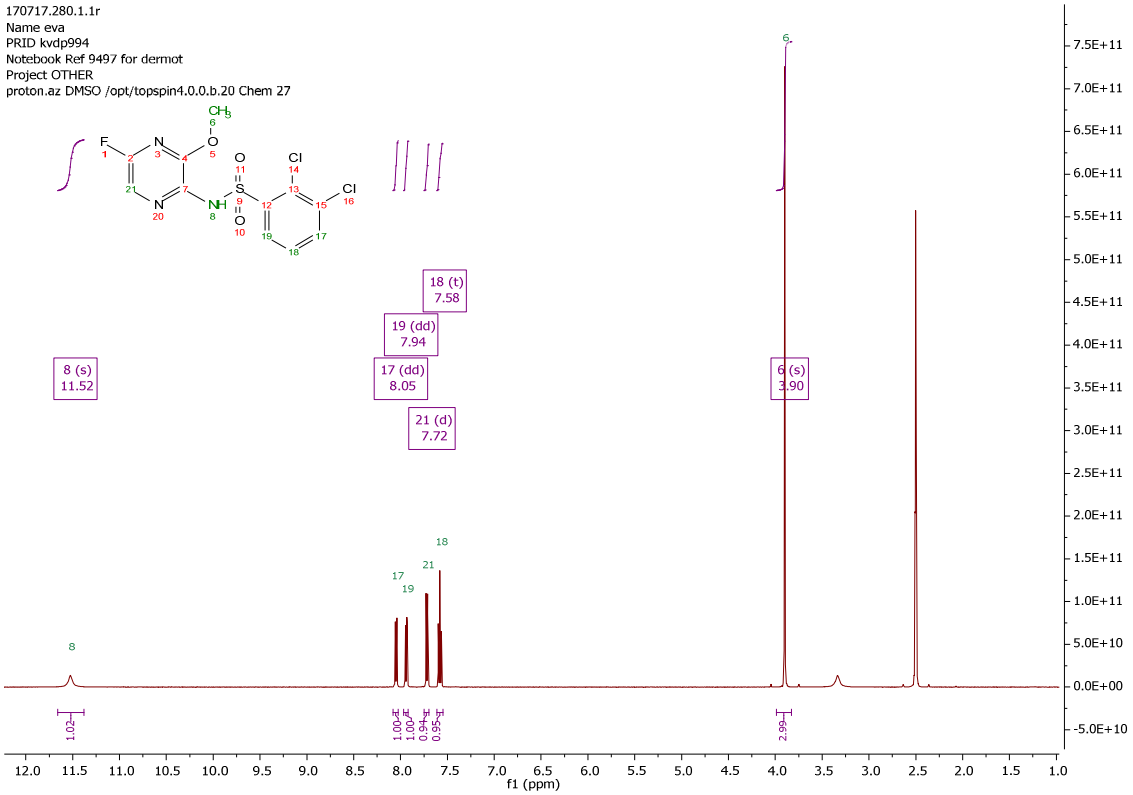
N-(5-Amino-3-methoxypyrazin-2-y1)-2,3-dichloro-benzenesulfonamide (**53**)

2,3-Dichloro-N-(3-methoxy-5-nitropyrazin-2-y1)-benzenesulfonamide (**52**) (4 g, 10.55 mmol) and 5% palladium on charcoal (Johnson Matthey type 440 paste) (0.8 g) in acetic acid (40 mL) was heated at 60°C under a hydrogen atmosphere (1 bar) until hydrogen uptake ceased (16 h). After cooling to room temperature the precipitated product and palladium catalyst was collected by filtration and washed with a little acetic acid. The solid was suspended in tetrahydrofuran (500 mL) and stirred for lh. The palladium catalyst was removed by filtration through celite. The tetrahydrofuran solution was evaporated to dryness and toluene added to the solid and evaporated under reduced pressure to give a light brown solid (2.6 g, 71%).
^{1}H NMR (D6-DMSO) δ 10.04 (1H, s), 7.91-7.88 (2H, m), 7.50 (1H, t), 7.08 (1H, s), 6.43 (2H, br s), 3.59 (3H, s).

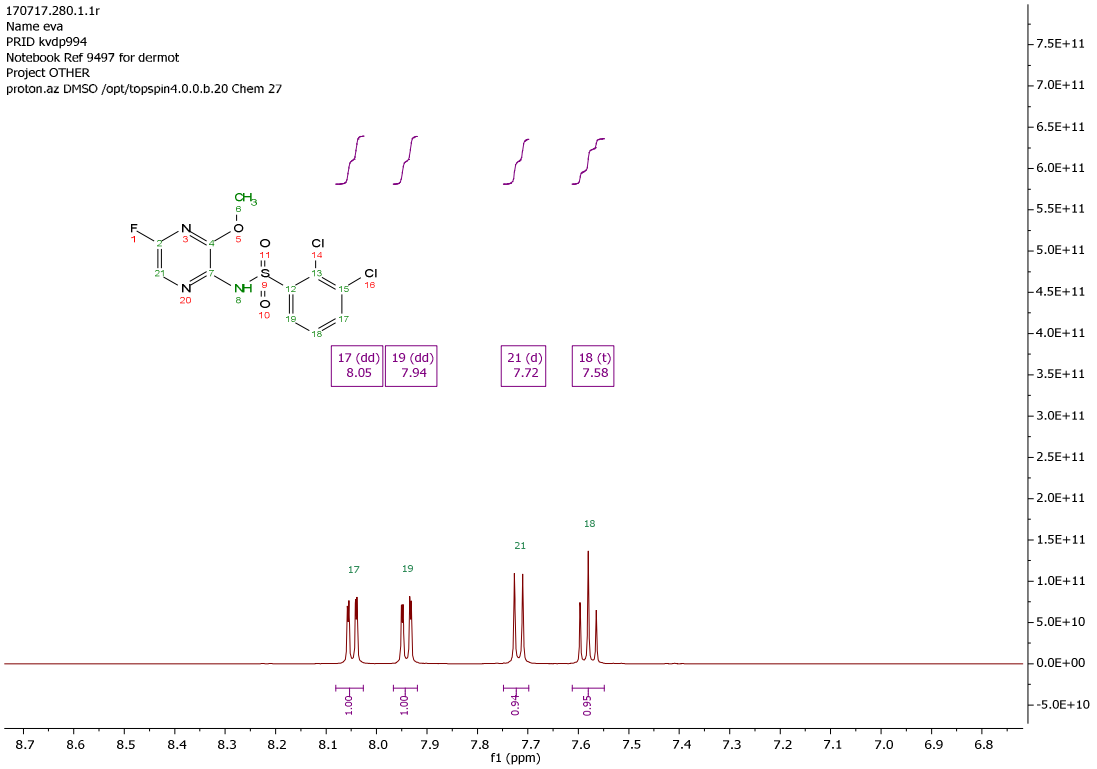
2,3-Dichloro-*N*-(5-fluoro-3-methoxypyrazin-2-yl)benzenesulfonamide (**49**).

Sodium nitrite (0.44 g, 6.4 mmol) was added portion wise to a stirred solution of N-(5-amino-3-methoxypyrazin-2-yl)-2,3-dichloro-benzenesulfonamide (**53**, 2 g, 5.7 mmol) in acetontrile (10 mL) and 48% aqueous HBF_4 (25 mL) cooled in an ice bath. After l h., the reaction mixture was poured on to water (250 mL) and extracted with ethyl acetate (3 x 25 mL). The ethyl acetate solution was dried ($MgSO_4$), filtered and evaporated to dryness and the product purified by silica gel chromatography eluting with ethyl acetate:iso-hexanes 1:4. The solvent was evaporated to afford the product (1.4 g, 70 %).
m.p. 151-152°C

LCMS (100% purity): m/e 350/352/354 $(M-H)^+$
^{1}H NMR (500 MHz, DMSO, 300K) 3.90 (s, 3H), 7.58 (t, *J* = 8.0 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.94 (dd, *J* = 1.5, 8.1 Hz, 1H), 8.05 (dd, *J* = 1.5, 8.0 Hz, 1H), 11.52 (s, 1H, NH).

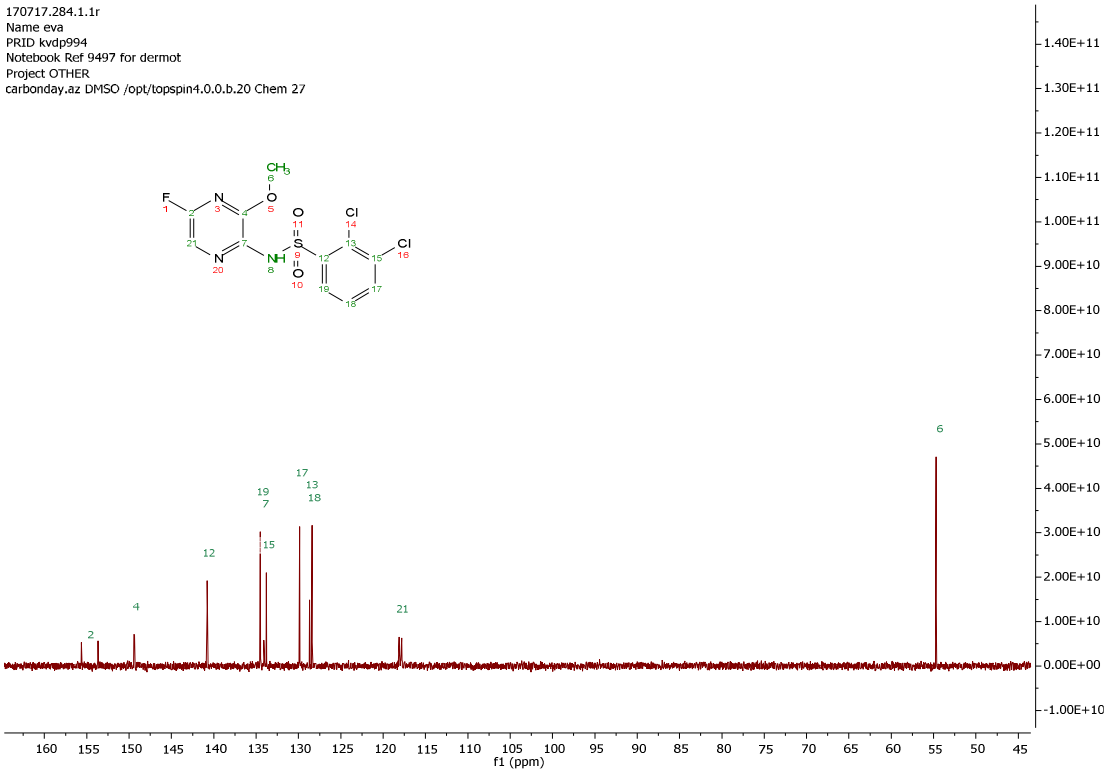


^{1}H NMR for compound **39** in DMSO

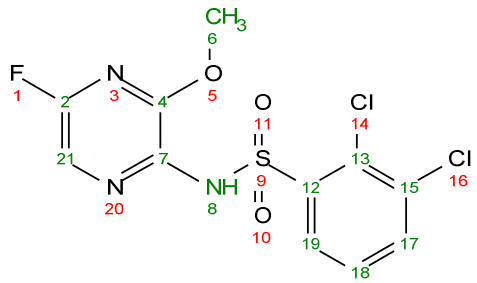


^{1}H NMR for compound **39** in DMSO

^{13}C NMR (126 MHz, DMSO, 300K) 54.71, 117.97 (J = 38.7), 128.39, 128.69, 129.87, 133.78, 134.08, 134.50, 140.79, 149.40 (J = 9.0), 154.66 (J = 248.7).



^{13}C NMR for compound **39** in DMSO



Atom No	^{1}H-Chemical Shift (ppm)	Multiplicity (*J* value)	^{13}C- Chemical Shift (ppm)
8	11.52	s	n/a NH
17	8.05	dd,*J*=8.01,1.53 Hz	129.87
19	7.94	dd,*J*=8.12,1.54 Hz	134.50
21	7.72	d,*J*=8.18 Hz	117.97
18	7.58	t,*J*=8.03 Hz	128.39
6	3.90	s	54.71
2			154.66
4			149.40
7			134.08
12			140.79
13			128.69
15			133.78

Pharmacological evaluation:

FMAT whole cell binding assay: CHO-K1 cells stably expressing the human recombinant CCR4 protein (Euroscreen) were cultured in NUT.MIX.F_12 (HAM) medium with glutamax-1, containing 10% (v/v) fetal bovine serum and 400 µgmL-1 geneticin. Cells were harvested at approximately 70% confluence by treatment with a cell dissociation buffer, and seeded at 5x103 cells/100µL and were incubated at 370C overnight in 5% CO2. The cell plates were washed with 100 µL Hanks balanced salt solution (HBSS). To each well was added HBSS (65 µL), 10 µL of DMSO in HBSS +/- test compound followed by FB-MDC (25 µL, 2.8 nM). After 2 hours incubation, the plates were analyzed in an FMAT8100 reader (Applied Biosystems) to measure fluorescence that was associated with binding of FB-MDC to the cells. Compound activity was determined as a pIC50 comparing fluorescence in control and background wells.