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Letter

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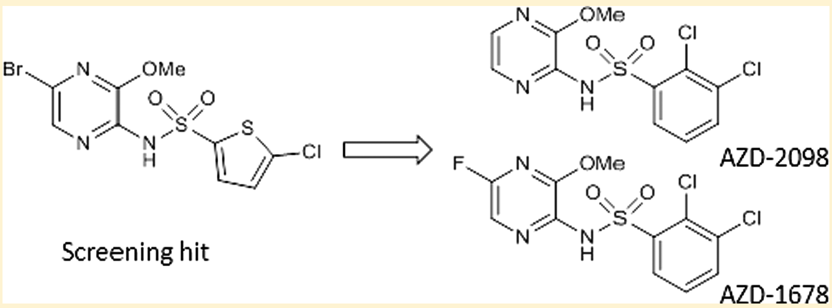
Discovery of AZD-2098 and AZD-1678, Two Potent and Bioavailable CCR4 Receptor Antagonists

Nicholas Kindon,[†,‡] Glen Andrews,[†] Andrew Baxter,[†] David Cheshire,[†] Paul Hemsley,[†,§] Timothy Johnson,[†] Yu-Zhen Liu,[†] Dermot McGinnity,[†,∥] Mark McHale,[†] Antonio Mete,[†] James Reuberson,[†] Bryan Roberts,[†] John Steele,[†,⊥] Barry Teobald,[†] John Unitt,[†] Deborah Vaughan,[†] Iain Walters,[†] and Michael J. Stocks*[,†,‡]

[†]AstraZeneca R&D Charnwood, Bakewell Road, Loughborough, LE11 5RH, U.K.

[⊥]Respiratory, Inflammation and Autoimmunity, Innovative Medicines and Early Development, AstraZeneca Gothenburg, Pepparedsleden 1, SE-431 83 Mölndal, Sweden

Supporting Information



ABSTRACT: *N*-(5-Bromo-3-methoxypyrazin-2-yl)-5-chlorothiophene-2-sulfonamide **1** was identified as a hit in a CCR4 receptor antagonist high-throughput screen (HTS) of a subset of the AstraZeneca compound bank. As a hit with a lead-like profile, it was an excellent starting point for a CCR4 receptor antagonist program and enabled the rapid progression through the Lead Identification and Lead Optimization phases resulting in the discovery of two bioavailable CCR4 receptor antagonist candidate drugs.

KEYWORDS: *Chemokine receptor 4, MDC, TARC, CCR4, antagonist*

The chemokine receptor family was originally identified in the 1990s and has since grown in number, complexity, and range of biological functions. Originally thought to be simple cell chemo attractants, chemokines have since been shown to exhibit a broader range of functions covering involvement in HIV-1 infection to hematopoiesis and control of cell growth.[1,2]

CCR4 is a G protein-coupled receptor (GPCR) and is activated by the CC chemokines, CCL22 (MDC: macrophage-derived chemokine) and CCL17 (TARC: T-cell and activation-related chemokine),[3] leading to cell activation and chemotaxis; it is expressed mainly by Th2 lymphocytes.[4,5] In keeping with a role for CCR4 in the orchestrated movement of Th2 cells into the allergic lung, both CCL17 and CCL22 have been shown to be elevated in the human lung following allergen challenge.[6] This potential role of CCR4 in driving human allergic lung disease led to many pharmaceutical companies attempting to identify CCR4 receptor antagonists for the treatment of allergic rhinitis and asthma,[7−15] but as yet, no drug has been discovered (Figure 1).[16,17]

In this communication, we are disclosing our studies on the optimization of *N*-(5-bromo-3-methoxypyrazin-2-yl)-5-chlorothiophene-2-sulfonamide **1** to afford two clinical candidates AZD-2098 (**47**) and AZD-1678 (**49**). High-throughput screening of the AstraZeneca Mixed Chemokine Receptor Antagonist Compound library (~29,000 compounds) using a *h*CCR4 fluorescent microvolume assay technology (FMAT) cell binding assay identified compound **1**[18,19] as an inhibitor of labeled CCL22 binding to CCR4 (pIC_{50} = 7.2).[20] Subsequently, functional antagonism of **1** was shown using an *h*CCR4 fluorescence imaging plate reader (FLIPR) intracellular calcium

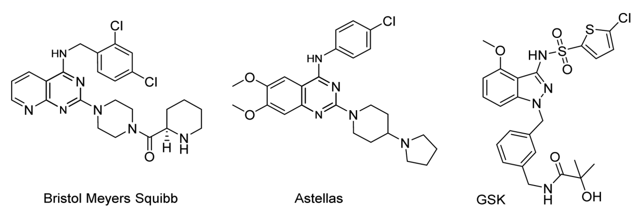


Figure 1. Structures of a selection of recently published CCR4 receptor antagonists.

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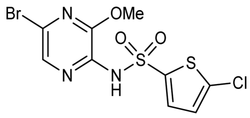
mobilization assay (pIC_{50} = 7.0) and a human primary Th2 cell chemotaxis assay (pIC_{50} = 6.7) (Table 1).

Table 1. Hit Profile of Pyrazine Sulfonamide 1



biological assay/test procedure	compound **1**
hCCR4 binding (pIC_{50})	7.2 ± 0.2
hPPB (%)	>99.9
calcd WBP (pIC_{50})[a]	<4.2
logD	1.1
solubility (mg/mL)	0.34
human hepatocytes (μL/mL/10^6 cells)	<0.2
rat/mouse in vivo PK	
Cl (mL/min/kg)	0.1/0.1
Vss (L/kg)	0.1/0.1
$T_{1/2}$ (h)	16/10
bioavailability (%)	45/86
rat/mouse CCR4 binding (pIC_{50})	8.3/8.3
rat/mouse PPB (%)	99.85/96.7

[a]Calculated from the *h*CCR4 binding and % age PPB.

Compound **1** exhibited a good lead-like profile[21] and was shown to be selective for the CCR4 receptor, through screening against an internal panel of chemokine receptors (e.g., CXCR1 and CXCR2, CCR1, CCR2b, CCR5, CCR7, and CCR8; inactive at 10 μM). When screened against a large panel of receptors and enzymes (~120 screens at MDS-Pharma), weak or no activity was observed and therefore **1** was chosen as a starting point for the CCR4 antagonist program. Compound **1** had good solubility (0.34 mg/mL), rat bioavailability (F = 45%), and half-life ($T_{1/2}$ = 16 h) when dosed in rat and a similar profile when dosed *in vivo* in mouse. This long half-life was accounted for by a very low clearance (0.1 mL/min/kg), which counteracts the small volume of distribution (Vss = 0.1 L/kg).

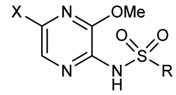
Compound **1** had very good crossover with rat and mouse CCR4 receptors (both pIC_{50} = 8.3). Subsequently, this species crossover was shown to be a general feature of the sulfonamide series (see Table 5), with the CCR4 receptor from all three species displaying very similar structure−activity relationships (SAR). When corrected for plasma protein binding, the predicted rat whole blood potency of **1** was pIC_{50} = 5.5 (rat ppb = 99.85%) and for mouse pIC_{50} = 6.8 (mouse ppb = 96.7%), giving **1** a suitable profile for use as a target validation tool for *in vivo* hypothesis testing.

However, the calculated human whole blood potency (WBP) was very poor with predicted activity (pIC_{50}) < 4.2 (calculated from *h*CCR4 pIC_{50} = 7.2 and *h*PPB > 99.9%).

With a candidate drug requiring a WBP $pIC_{50} \approx 6.0$, the key issue was to increase WBP by a combination of increasing CCR4 receptor affinity and lowering plasma protein binding,[22] while maintaining all the other good features inherent in **1**. The high plasma protein binding was attributed to the acidic sulfonamide-NH (measured pK_a = 4.1) and the lipophilic nature of the molecule (measured logP = 4.4).

The SAR generated with respect to modification of the 5-chlorothiophene group is shown in Table 2. Removal of the 5-chloro substituent **2** gave over a 10-fold drop in affinity, as did the isomeric unsubstituted thiophene **3**. Addition of a chlorine to the 4-position of the thienyl group also gave a reduction in affinity

Table 2. Exploration of the 5-Position of the Pyrazine Ring



example	R	X	CCR4 pIC_{50}[a]
1	5-chloro-2-thienyl	Br	7.2 ± 0.2
2	2-thienyl	Br	6.2 ± 0.2
3	3-thienyl	Br	5.9 ± 0.1
4	4,5-dichloro-2-thienyl	Br	6.0 ± 0.1
5	3-bromo-5-chloro-2-thienyl	Br	7.4 ± 0.2
6	3-phenyl-2-thienyl	Br	6.0 ± 0.1
7	phenyl	Br	6.1 ± 0.1
8	2-chlorophenyl	Br	6.8 ± 0.2
9	3-chlorophenyl	Br	7.0 ± 0.2
10	4-chlorophenyl	Br	6.9 ± 0.2
11	2,6-dichlorophenyl	Br	6.2 ± 0.2
12	2,5-dichlorophenyl	Br	5.5 ± 0.1
13	2,4-dichlorophenyl	Br	6.9 ± 0.2
14	3,5-dichlorophenyl	Br	5.3 ± 0.1
15	3,4-dichlorophenyl	Br	6.7 ± 0.2
16	2,3-dichlorophenyl	Br	8.0 ± 0.2
17	2,3-dichlorophenyl	Cl	7.9 ± 0.2
18	3,4-dichloro-2-thienyl	Cl	8.1 ± 0.2
19	2-chloro-3-fluorophenyl	Cl	7.8 ± 0.2
20	3-chloro-2-fluorophenyl	Cl	7.1 ± 0.2
21	3-chloro-2-methylphenyl	Cl	7.7 ± 0.2
22	2-chloro-3-(trifluoromethyl)phenyl	Cl	7.2 ± 0.1
23	3-chloro-2-cyanophenyl	Cl	7.2 ± 0.2
24	3-chloro-2-methylthiophenyl	Cl	7.1 ± 0.2
25	2,3-dichloro-4-pyridyl	Cl	5.0 ± 0.1
26	butyl	Cl	5.5 ± 0.2

[a]Potency is given as pIC_{50} values with $n = \geq 2$ replicates.

(compare **4** with **1**), while a large substituent (bromine or phenyl) in the 3-position was tolerated (compare **5** and **6** with **1** and **2**, respectively). The phenyl analogue **7** was similar to the two unsubstituted thiophenes in potency, and introducing chlorine into the 2-, 3- or 4-positions of the phenyl sulfonamide ring (**8**, **9** and **10**) gave an increase in binding relative to **7**. The 6-substituted dichlorophenyl sulfonamides (**11**−**17**) had a potency range of almost 3 log units, with the 3,5-dichlorophenylsulfonamide analogue **14** having only weak activity (pIC_{50} = 5.3) and the 2,3-dichlorophenylsulfonamide **16** being optimal (pIC_{50} = 8.0), almost a log unit more potent than **1**. Similar affinity was achieved with 3,4-dichloro-2-thienyl (compare **18** and **17**). A number of other disubstituted phenyl sulfonamides were screened; however, no improvement in binding was seen (**19**−**24** compare with **17**). Introduction of a nitrogen into the aromatic ring led to dramatic reduction in potency (**25**) as did replacement of the aromatic ring with an alkyl group as illustrated with **26**.

While an increase in affinity was achieved with compounds **16** and **17**, changing the 5-chlorothiophene group for 2,3-dichlorophenyl did not significantly change the lipophilicity. Consequently, the *h*PPB figures for these two compounds was also high (>99.9%).

The exploration of the 3-position is shown in Table 3. Initially the atom linking the 3-substituent to the pyrazine ring was investigated. Changing methoxy for methylthio, methylamino, or ethyl (**28**, **29**, and **30**) gave a large drop in potency, establishing oxygen as the optimal atom with which to attach the 3-substituent to the pyrazine ring. Next, a small number of 3-alkoxy

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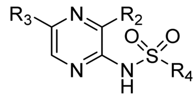
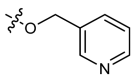
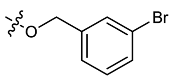
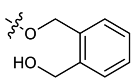
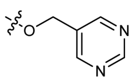
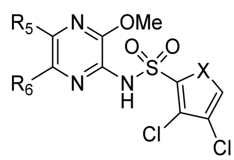
Table 3. Exploration of the 3-Position of the Pyrazine Ring



Example	R_2	R_3	R_4	CCR4 receptor antagonism (pIC_{50})[a]
27	OEt	Br	5-Chloro-2-thienyl	6.7 ± 0.2
28	SMe	Br	5-Chloro-2-thienyl	5.5 ± 0.2
29	NHMe	Br	5-Chloro-2-thienyl	<5.0*
30	Et	Br	5-Chloro-2-thienyl	5.3 ± 0.2
31	OBu	Cl	2,3-Dichlorophenyl	6.8 ± 0.1
32	$OCH_2{}^tBu$	Cl	2,3-Dichlorophenyl	5.1 ± 0.2
33	OCH_2CHCH_2	Cl	2,3-Dichlorophenyl	7.8 ± 0.1
34	OCH_2CCH	Cl	2,3-Dichlorophenyl	8.6 ± 0.2
35		Cl	2,3-Dichlorophenyl	9.2± 0.3
36		Cl	2,3-Dichlorophenyl	8.4 ± 0.2
37		Cl	2,3-Dichlorophenyl	9.5 ± 0.2
38		Cl	2,3-Dichlorophenyl	7.9 ± 0.3

[a]Potency is given as pIC_{50} values with $n = \geq 2$ replicates. *44% inhibition at 10 μM.

Table 4. Exploration of the 5- and 6-Position of the Pyrazine Ring



example	X	R_5	R_6	CCR4 receptor antagonism (pIC_{50})[a]	LogP	hPPB (%)	calcd WBP (pIC_{50})
17	CH=CH	Cl	H	7.9 ± 0.2	4.1	>99.9	
39	CH=CH	H	Cl	8.1 ± 0.3	4.5	>99.9	
40	CH=CH	OEt	H	7.9 ± 0.2	4.1	>99.9	
41	CH=CH	SO_2Me	H	5.3 ± 0.2			
42	CH=CH	Me	H	7.4 ± 0.2	3.0	99.65	4.9
43	CH=CH	H	Me	7.3 ± 0.3		>99.8	
44	CH=CH	CH_2OH	H	7.6 ± 0.2	2.1	96.2	6.2
45	CH=CH	CH_2OH	Cl	8.4 ± 0.2	3.2	99.6	6.0
46	CH=CH	CH_2OH	Me	7.4 ± 0.1	2.4	98.7	5.5
47	CH=CH	H	H	7.8 ± 0.05	2.5	98.9	5.8
48	S	H	H	7.7 ± 0.2	2.8	97.5	6.1
49	CH=CH	F	H	8.6 ± 0.1	3.1	99.4	6.4
50	CH=CH	H	F	8.4 ± 0.2	3.3	>99.8	
51	CH=CH	F	F	9.0 ± 0.3		>99.8	

[a]Potency is given as pIC_{50} values with $n = \geq 2$ replicates.

analogues were synthesized (**27**, **31**, and **32**). In each case, there was a substantial drop in potency relative to the parent methoxy-substituted compound (**1** or **17**). However, the 3-allyloxy analogue (**33**) maintained activity and with the 3-propargyloxy (**34**) analogue affording a slight increase in activity. Further increases in activity were obtained with 3-OCH_2Aryl and 3-OCH_2heteroaryl (**35**, **36**, **37**, and **38**), where substitution gave substantial increase in activity (pIC_{50} up to 9.5). Although this SAR demonstrated how to improve on CCR4 affinity, the increase in lipophilicity associated with these changes did not address the plasma protein binding issue with *h*PPB > 99.9% in each case.

In contrast to the 3-position, the exploration of the 5- and 6-positions on the pyrazine ring allowed for the discovery of

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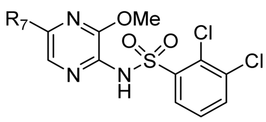
compounds with increased activity combined with lower lipophilicity relative to **17** (Table 4).

A wide variety of substituents were tolerated in the 5- and 6-positions, and it was discovered that the percentage plasma protein binding was reduced by lowering the lipophilicity of the compounds, affording plasma protein binding below 99% for compounds of modest lipophilicity (logP < 3.0). For **44**, **45**, **47**, **48**, and **49**, the calculated WBP (pIC_{50}) of 5.8−6.4 was achieved. These met the candidate drug target profile set of WBP $pIC_{50} \approx 6.0$. Of these four compounds, **47** and **49** had the best overall profile for progression (Table 5). Compounds **47** and **49** demonstrated good selectivity for the CCR4 receptor when screened in-house against a range of other chemokine receptors (CXCR1 and CXCR2, CCR1, CCR2b, CCR5, CCR7, and CCR8; all inactive at 10 μM) and little or no activity when screened against a large panel of receptors and enzymes (∼120 screens at MDS-Pharma). A pharmacokinetic profile commensurate with once-daily oral administration enabled both compounds to progress as candidate drugs.[23]

Table 5. Compound Profiles of 47 and 49



screen	**47** (R^7 = H)	**49** (R^7 = F)
hCCR4 pIC_{50}	7.8 ± 0.05 (n = 11)	8.6 ± 0.1 (n = 10)
hPPB (%)	98.9	99.4
calcd WBP (pIC_{50})	5.9	6.4
logD	0.35	0.6
solubility (mg/mL)	1.5	1.3
human hepatocytes (μL/mL/10^6 cells)	<0.2	<1.0
Species Cross Over		
rat CCR4 pIC_{50}	8.0 ± 0.27 (n = 3)	9.0 ± 0.1 (n = 3)
mouse CCR4 pIC_{50}	8.0 ± 0.04 (n = 3)	9.0 ± 0.1 (n = 3)
dog CCR4 pIC_{50}	7.6 ± 0.06 (n = 7)	8.5 ± 0.2 (n = 3)
Rat/Dog in Vivo PK[a]		
Cl (mL/min/kg)	2.0/1.5	2.3/3.7
Vss (L/kg)	0.2/0.1	0.2/0.2
$T_{1/2}$ (h)	1.7/1.0	2.5/0.5
F (%)	100/100	100/100
dose-to-man prediction (mg/kg) UID	1	0.1

[a]PK studies conducted using 1% sodium bicarbonate solution at 9.0 μmol/kg (p.o.) or 3.0 μmol/kg (i.v.).

The antagonist potency of **47** and **49** was assessed in a number of cell systems in which a response mediated by the human CCR4 receptor can be evoked by MDC or TARC and quantified using changes in intracellular calcium concentration or chemotaxis. The cell systems used were a cell line (CHO) transfected with the human CCR4 receptor and human CD4+, CD45RA+ Th2 cells in 0.3% HSA. Findings are summarized in Table 6. The reduced potency in the presence of added protein is consistent with the drop-off predicted from plasma protein binding measurements. Both **47** and **49** demonstrated no agonist activity at concentrations up to 10 μM.

The anti-inflammatory effects of **47** were investigated in Brown−Norway rats sensitized to ovalbumin. Sensitized rats were dosed orally (BID) with **47** at 0.22, 0.75, 2.2, 3.0, 7.5, and 15 μmol/kg 1 h prior to antigen challenge and every 12 h thereafter prior to termination 96 h post-challenge. Histopathological examination of the lung tissue showed a marked, dose-dependent reduction in a range of histological correlates with reduced alveolitis and leukocyte trafficking in the microvasculature being the most diagnostic features of efficacy. The changes were first visible at a dose of 0.22 μmol/kg and maximal at 7.5 μmol/kg. Plasma samples were taken 12 h after the last dose of compound for measurement of terminal trough concentrations of **47** to demonstrate a strong PKPD correlation (Table 7).

Table 7. Terminal Plasma Concentrations of 47

dose (μmol/kg)	total plasma concentration (μM)	free plasma concentration /pIC_{50}
0.22	<LOQ[a]	
0.75	<LOQ[a]	
2.2	<LOQ[a]	
3.0	0.033 ± 0.018	0.6
7.5	0.14 ± 0.069	3
15	0.49 ± 0.18	9

[a]LOQ was 0.03 μM for the 0.22, 0.75, and 2.2 μmol/kg doses.

The synthesis of **47** and **49** is shown (Scheme 1). Dibromination of 2-aminopyrazine followed by selective displacement of the bromine in the 3-position with sodium methoxide gave 2-amino-5-bromo-3-methoxypyrazine. The bromine in the 5-position was then removed by hydrogenation and coupling of the 2,3-dichlorophenylsulphonyl chloride with 2-amino-3-methoxypyrazine afforded **47**. A highly selective 5-position nitration of **47** followed by reduction of the nitro group **52** gave an amino-pyrazine **53** that was diazotized in the presence of hydrofluoric acid to give **49**.

In summary, a new series of potent and bioavailable amino-pyrazine sulfonamide CCR4 receptor antagonists have been developed from a lead optimization program initiated from a high-throughput screening/synthesis campaign. The initial hit series proved to have reasonable activity but was hindered by having very high human plasma protein binding resulting in a low free fraction of compound. Through chemical modification, both the potency of the series and the free fraction were enhanced to ultimately deliver two clinical candidates. Further studies on these compounds will be reported in due course.

Table 6. Potency of 47 and 49 for Inhibition of Cellular Responses Mediated by the Human CCR4 Receptor

cell system	assay readout	**47** (pIC_{50} mean ± SEM)	**49** (pIC_{50} mean ± SEM)
inhibition of CCL22 Ca^{2+} response	MDC-induced Ca^{2+} flux	7.5 ± 0.04 (n = 3) (CHO cells expressing hCCR4)	8.4 ± 0.1 (n = 4) (HEK cells expressing hCCR4)
inhibition of Th2 cell CCL22 driven chemotaxis in 0.3% HSA	MDC-induced chemotaxis	6.3 ± 0.2 (n = 3)	6.8 ± 0.2 (n = 3)
inhibition of Th2 cell CCL17 driven chemotaxis in 0.3% HSA	TARC-induced chemotaxis	6.3 ± 0.1 (n = 3)	6.5 (n = 1)

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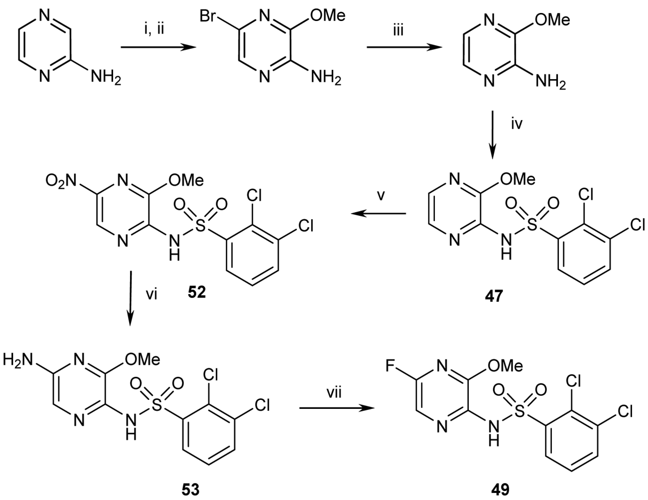
Scheme 1[a]



[a]Reagents and conditions: (i) Br_2, dichloromethane, 2,6-lutidine (85-95%); (ii) NaOMe (3 equiv), MeOH, reflux (90-95%); (iii) H_2, Pd/C, ethanol, rt (95%) (iv) $ArSO_2Cl$, KO*t*Bu, 0–25°C, THF (80–95%); (v) HNO_3, AcOH, 70–85 °C (75-82%); (vi) H_2, Pd/C, AcOH, 60 °C (75-92%); (vii) HBF_4, CH_3CN, 0–5 °C, $NaNO_2$ (40%) or HF/pyridine/$NaNO_2$ (85%). Range of percentage yields across multiple reactions and scale (~100 mg to >50 g).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00315.

Representative synthetic procedures and analytical data for **47** and **49**. Description of biological assay (CCR4 receptor antagonism using FMAT whole cell binding) (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: michael.stocks@nottingham.ac.uk.

ORCID

Michael J. Stocks: 0000-0003-3046-137X

Present Addresses

[‡]School of Pharmacy, Centre for Biomolecular Sciences, The University of Nottingham, University Park, Nottingham, NG7 2RD, U.K.

[§]Discovery Sciences, Innovative Medicines & Early Development, AstraZeneca, Darwin Building, Cambridge Science Park, Cambridge, CB4 0FZ, U.K.

[∥]IMED Oncology, Innovative Medicines & Early Development, AstraZeneca, Chesterford Research Park, Cambridge, CB10 1XL, U.K.

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CCR4, CC chemokine receptor 4; CCL12, CC chemokine ligand 12; F, bioavailability; FLIPR, fluorescence imaging plate reader; FMAT, fluorescent microvolume assay technology; GPCR, G protein-coupled receptor; MDC, macrophage-derived chemokine; PPB, plasma protein binding; TARC, T-cell and activation-related chemokine; UID, once-a-day; Vss, volume of distribution at steady state

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