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Synthesis and Structure–Activity Relationships of Indazole Arylsulfonamides as Allosteric CC-Chemokine Receptor 4 (CCR4) Antagonists

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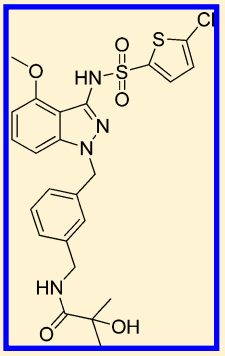
Supporting Information

ABSTRACT: A series of indazole arylsulfonamides were synthesized and examined as human CCR4 antagonists. Methoxy- or hydroxyl- containing groups were the more potent indazole C4 substituents. Only small groups were tolerated at C5, C6, or C7, with the C6 analogues being preferred. The most potent *N*3-substituent was 5-chlorothiophene-2-sulfonamide. *N*1 *meta*-substituted benzyl groups possessing an α-amino-3-[(methylamino)acyl]− group were the most potent *N*1-substituents. Strongly basic amino groups had low oral absorption in vivo. Less basic analogues, such as morpholines, had good oral absorption; however, they also had high clearance. The most potent compound with high absorption in two species was analogue **6** (GSK2239633A), which was selected for further development. Aryl sulfonamide antagonists bind to CCR4 at an intracellular allosteric site denoted site II. X-ray diffraction studies on two indazole sulfonamide fragments suggested the presence of an important intramolecular interaction in the active conformation.



INTRODUCTION

Chemokines are a group of small, basic proteins of 8−10 kDa, which together with their receptors mainly regulate the trafficking of leucocytes down a chemoattractant gradient.[1,2] Chemokines possess four conserved cystein residues, and they are classified into four groups designated CC, CXC, C, and CX_3C based on the arrangement of the first two conserved cysteine residues located at or near the *N*-terminus of each protein.[3] Ten CC chemokine receptors have been identified so far and named as CC-chemokine receptor 1, 2, 3, etc. Most chemokine receptors recognize more than one chemokine and several chemokines bind to more than one receptor.[1] CCR4 belongs to the 7-TM domain G-protein-coupled receptor family and is mainly expressed in T helper 2 (Th2) cells. The latter are a subset of CD4-positive T helper cells that produce interleukin (IL)-4, IL-5, and IL-13.[4] Th2 cytokines in inflamed tissues lead to eosinophilia, high levels of serum IgE, and mast cell activation, all of which contribute to the pathogenesis of allergic diseases.[5] CC Chemokine ligand 17 (CCL17), previously known as thymus activation-regulated chemokine (TARC), and CC chemokine ligand 22 (CCL22), also known as macrophage-derived chemokine (MDC), bind to the orthosteric binding site of CC-chemokine receptor 4 (CCR4).[3] Upon exposure to allergen, dendritic cells within the lung (or other tissue) secrete MDC and TARC (also produced by endothelial cells) which can recruit Th2 cells from the circulation. The T cells can then migrate along this chemokine gradient to the dendritic cells. Upon maturation, the dendritic cells migrate from the inflamed tissue to local lymph nodes where the MDC and TARC which they produce may recruit further T cells to the inflammatory response. Elevated levels of TARC and MDC as well as accumulation of CCR4-positive cells have been observed in lung biopsy samples from patients with atopic asthma following allergen challenge.[6,7] Thus CCR4 antagonists represent a novel therapeutic intervention in diseases where CCR4 has a central role in pathogenesis, such as asthma, atopic dermatitis,[8] allergic bronchopulmonary aspergillosis,[9] cancer,[10] the mosquito-borne tropical diseases, such as Dengue fever,[11] and allergic

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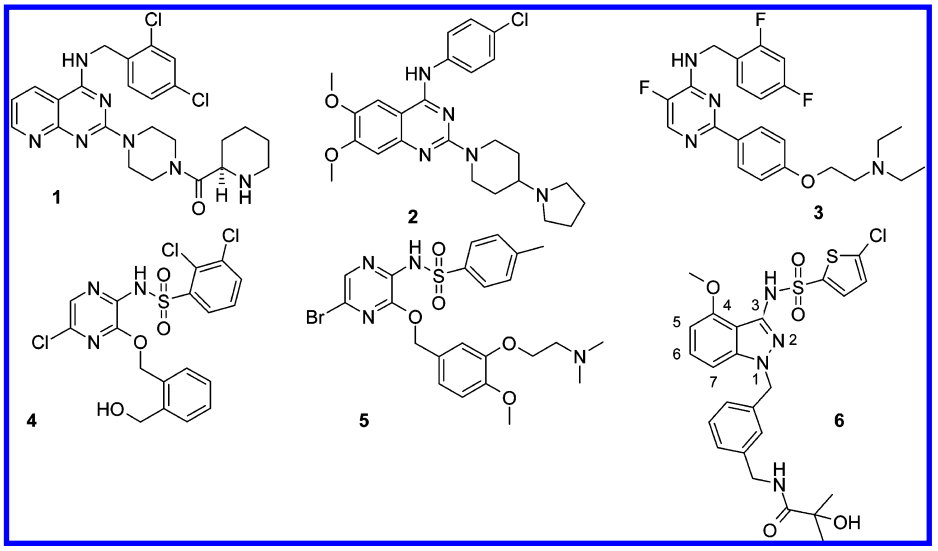
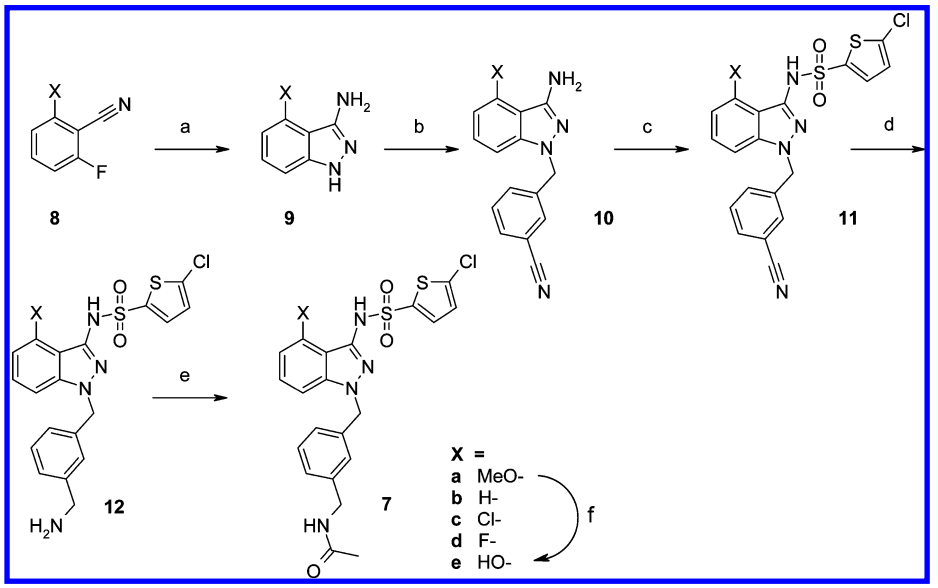


Figure 1. Structures for some recently published CCR4 antagonists.

Scheme 1[a]



[a]Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, 1-butanol, reflux, 80−92%; (b) KOH, DMSO, 3-cyanobenzyl chloride, 52−84%; (c) 5-chloro-2-thiophenesulfonyl chloride, pyridine, 29−87%; (d) 1 M $LiAlH_4$ solution in ether, THF, 55−77%; (e) Ac_2O, Et_3N, DCM, 38−89%; (f) BBr_3, DCM, 33%.

rhinitis.[12] Progress in the discovery of small-molecule CCR4 antagonists as immunomodulatory agents was reviewed by Purandare and Somerville in 2006.[13] A number of publications on CCR4 antagonists have appeared in the literature since the last review,[14−23] and these antagonists appear to belong to two chemical categories (Figure 1). The first category includes lipophilic heteroarenes possessing basic amino groups such as Bristol Myers Squibb (BMS) compound **1**,[15] Astellas compound **2**,[18] and the Daiichi Sankyo compound **3**.[22] The AstraZeneca (AZ) 2,3-dichlorobenzenesulfonamide **4**[24] and Ono 4-methylbenzenesulfonamide **5**[25] belong to the second category of pyrazine arylsulfonamides. More recently, we have disclosed our own preliminary studies on a novel class of indazole arylsulfonamides including **6**, which we believe is the first small-molecule clinical candidate targeting the CCR4 receptor.[26]

The AstraZeneca group have reported the discovery of a novel mechanism for antagonism of CCR4 and CCR5 receptors with a series of small-molecule pyrazine sulfonamides, which interact with an intracellular allosteric site on the receptor.[27] The precise location of this allosteric binding site was not determined, however, it was suggested that it might be a generic site for chemokine receptors, or even more broadly for class A G-protein-coupled receptors. In our recent communication disclosing sulfonamide **6**, we focused our investigations entirely on the variation of the indazole *N*1 substituent.[26] In this publication, we present a fuller account of the structure−activity relationships (SAR) of the indazole arylsulfonamides covering the C4, C5, C6, C7, *N*3-sulfonamide, and additional *N*1-substitutions, together with extensive in vivo pharmacokinetic studies on 10 analogues, and shed light on the spatial requirement of the sulfonamide antagonists based on small-molecule X-ray diffraction studies.

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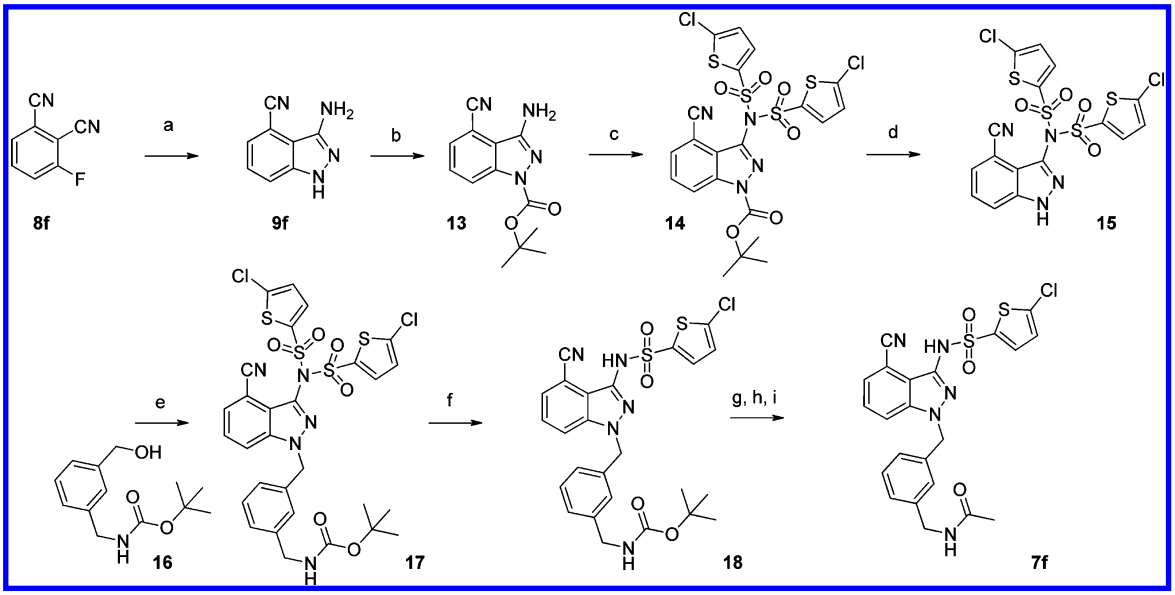
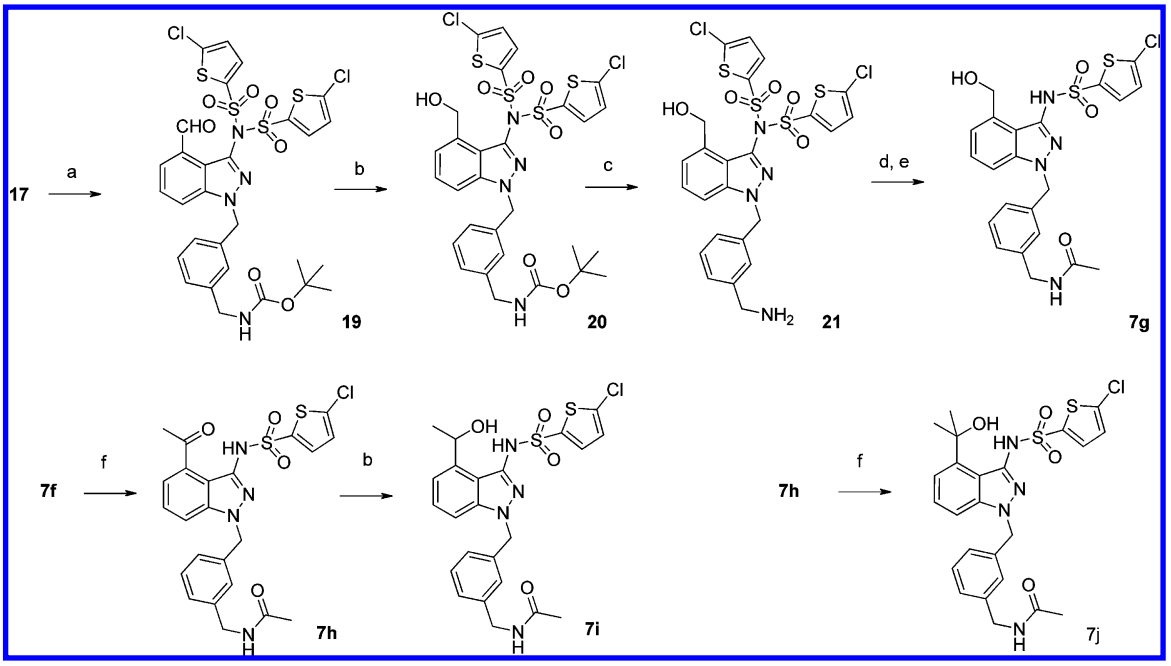
Scheme 2[a]



[a]Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, EtOH, 70 °C, 87%; (b) BOC_2O, DMAP, Et_3N, MeCN, 56%; (c) 5-chloro-2-thiophenesulfonyl chloride, DCM, pyridine, 56%; (d) TFA, DCM, 43%; (e) *N*-BOC-3-(hydroxymethyl)benzylamine (**16**), PPh_3, $^iPrO_2CN{=}NCO_2Pr^i$, THF, 60 °C, 96%; (f) 2 M NaOH, MeOH, THF, 45 °C, 1 h; (g) TFA, DCM; (h) Ac_2O, Et_3N, DCM; (i) K_2CO_3, MeOH, 19%.

Scheme 3[a]



[a]Reagents and conditions: (a) DIBAL-H, PhMe; (b) $NaBH_4$, MeOH, 17–48%; (c) TFA, DCM, 66%; (d) Ac_2O, Et_3N, DCM; (e) 2 M aq NaOH, MeOH, 20 °C, 43%; (f) MeMgBr, THF, 30–77%.

CHEMISTRY

The SAR investigations commenced with examination of the C4 substituted indazoles **7** outlined in Scheme 1. Reaction of the appropriately substituted 2-fluoro-benzonitrile **8a–d** with hydrazine hydrate in *n*-butanol gave the amino indazoles **9a–d**, which were selectively alkylated with 3-cyanobenzyl chloride in DMSO in the presence of powdered KOH to provide **10a–d**. This procedure to introduce the *N*1 substituent was an improvement over our previous protocol which required the use of protecting group chemistry.[26] Reaction of **10a–d** with 5-chloro-2-thiophenesulfonyl chloride in pyridine gave the sulfonamides **11a–d**, the cyano group of which was reduced with $LiAlH_4$ to give the benzylamines **12a–d**. Acetylation of **12a–d** with acetic anhydride gave the required amides **7a–d**. On some occasions, **7** was accompanied by formation of a small amount of diacetylated products, which were converted back to **7** by trans-esterification with methanol over anhydrous potassium carbonate prior to workup. The phenolic analogue **7e** was prepared from **12a** by treatment with boron tribromide to give **12e** (33%), followed by acetylation with acetic acid in

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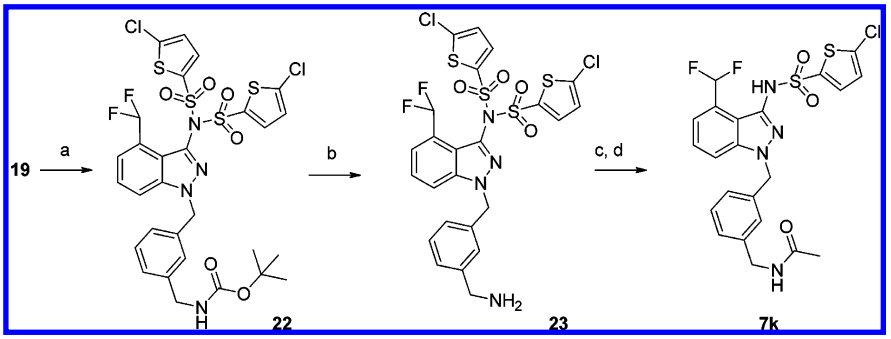
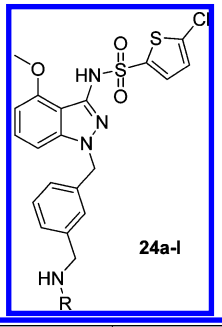
Scheme 4[a]



[a]Reagents and conditions: (a) DAST, DCM, 5 h, 15%; (b) TFA, DCM, 54%; (c) Ac_2O, Et_3N, DCM; (d) 2 M aq NaOH, MeOH, 60 °C, 3 h, 36%.

Table 1



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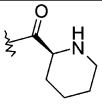
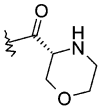
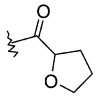
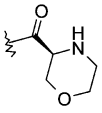
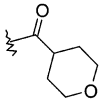
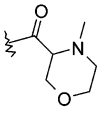
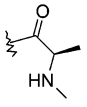
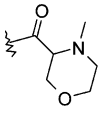
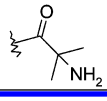
a		g	
b		h	
c		i	
d		j	 isomer A
e		k	 isomer B
f		l	

the presence of HATU and diisopropylethylamine in DMF (69%).

The 4-cyano analogue **7f** was prepared by the route outlined in Scheme 2. Reaction of 3-fluoro-1,2-benzenedicarbonitrile (**8f**) with hydrazine hydrate in ethanol gave the 3-amino-indazole **9f** (87%), which was then protected with di-*tert*-butyl dicarbonate in the presence of DMAP and Et_3N in MeCN to give **13** in 56% yield. Treatment of **13** with excess 5-chloro-2-thiophenesulfonyl chloride gave the bis-sulfonamide **14** in 56% yield, which was deprotected with TFA to give **15** in 43% yield. Mitsunobu reaction of **15** with *N*-BOC-3-(hydroxymethyl)-benzylamine[28] (**16**) gave **17** (96%), which was first hydrolyzed with NaOH to give the monosulfonamide **18**, then deprotected with TFA, acetylated with Ac_2O, and finally treated with potassium carbonate in MeOH to cleave overacetylated product, and provide **7f**.

The C4-hydroxymethyl analogue **7g** was obtained by DIBAL-H reduction of the nitrile **17**, followed by $NaBH_4$ reduction of the resulting aldehyde **19** to give **20**, cleavage of the *N*-BOC protecting group with TFA to give the benzylamine

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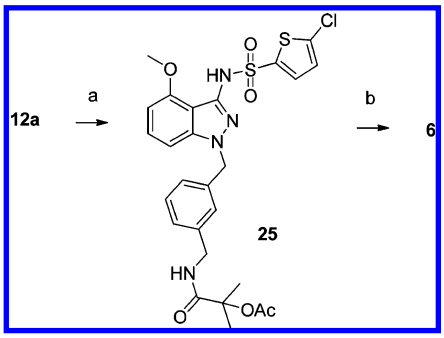
21, followed by acetylation with Ac_2O, and finally hydrolysis with NaOH (Scheme 3). The C4-acetyl analogue **7h** was prepared by methyl magnesium bromide addition to the nitrile **7f** (77%), and the secondary alcohol **7i** was prepared by $NaBH_4$ reduction of **7h** (48%). Addition of MeMgBr to ketone **7h** gave the tertiary alcohol **7j** (30%).

Scheme 4 outlines the preparation of the C4-difluoromethyl analogue **7k** which was prepared from the aldehyde **19** using diethylamino-sulfur trifluoride (DAST) in DCM to give **22** in 15% yield. The latter was deprotected with TFA to give the amine **23** (54%), which was then acetylated and deprotected to give **7k** in 36% yield for the two steps.

Further amide analogues of **6** containing either an ether (**a**–**d**) or amino (**e**–**k**) group were prepared by acylation of **12a** with the appropriate carboxylic acid in DMF in the presence of *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride, *N*-hydroxybenztriazole hydrate (HOBT), and *N*-methylmorpholine as base to give amides **24a**–**k** (Table 1). The amino-acids (**e**–**i**) used in the amide coupling reactions were *N*-BOC protected and were subsequently deprotected with either 4 M HCl in dioxane or with TFA in DCM. The *N*-methyl-morpholine enantiomers **24j** and **24k** were made from the racemic amino acid and separated by preparative chiral HPLC. The two enantiomers were labeled isomer A and B as their absolute configuration was not determined. Compound **24l** was prepared from **12a** and 3-oxetanone in the presence of sodium triacetoxyborohydride in THF in 94% yield.

Compound **6** was prepared on a larger scale by reaction of **12a** with 2-acetoxy-isobutyryl chloride in DCM and Et_3N to give **25** in 77% yield, followed by trans-esterification of the acetate ester with methanol over anhydrous K_2CO_3 (71%) (Scheme 5).

Scheme 5[a]



[a]Reagents and conditions: (a) 2-acetoxy-isobutyryl chloride, Et_3N, DCM, 77%; (b) K_2CO_3 (3 equiv), MeOH, 2 h, 20 °C, 71%.

The three pyridyl analogues of **7a**, regioisomers **26**, **27**, and **28**, were prepared from the bis-sulfonamide **29**[28] according to Scheme 6, which outlines only the synthesis of regioisomer **26**. Thus Mitsunobu coupling of **29** with 2-(hydroxymethyl)-4-pyridinecarbonitrile **30**[29] gave **31**, which was reduced with $LiAlH_4$ and hydrolyzed with aqueous NaOH in MeOH to give the benzylamine **32**. The latter was acetylated with AcOH in the presence of HATU and Et_3N to give **26**. The other two regioisomers **27** and **28** were synthesized by an analogous method starting from **29** and the appropriate hydroxymethyl-pyridinecarbonitrile.

The 5-fluoro-analogues **33a** and **33b** were prepared by the route shown in Scheme 7. Reaction of 3,6-difluoro-2-methoxybenzonitrile (**34**) with hydrazine monohydrate in EtOH gave **35** in 25%. The latter was reacted with 3-chloromethyl benzonitrile in the presence of powdered KOH in DMSO to give **36**, which was reduced with sodium borohydride in the presence of nickel(II) chloride and di-*tert*-butyl dicarbonate in THF and MeOH to give **37**. Sulfonylation of **37** gave **38**, which was then deprotected with TFA in DCM to give the amine **39**. Acylation of **39** with AcOH or 2-acetoxy-isobutyryl chloride and hydrolysis gave the two amides **33a** and **33b**, respectively.

Scheme 8 outlines the synthesis of the 6-fluoro analogues **40a** and **40b**. Treatment of 3,5-difluoroanisole with α,α-dichloromethyl methyl ether in the presence of $TiCl_4$ gave the aldehyde **42**, which was converted to the nitrile **43** with hydroxylamine-*O*-sulfonic acid and then cyclized to the indazole **44**. Alkylation with 3-chloromethylbenzonitrile gave **45**, which was converted to the sulfonamide **46** and then reduced with $LiAlH_4$ to give the amine **47**. The latter was acetylated to **40a** and also converted to the amide **40b** as previously.

The 7-fluoro analogue **48** was obtained by the route outlined in Scheme 9. Treatment of 2,3-difluoro-6-methoxybenzonitrile **49** with hydrazine hydrate in *N*-methylpyrrolidinone (NMP) gave the indazole **50**, which was alkylated with 3-chloromethylbenzonitrile as before to provide **51**. The latter was reacted with 5-chloro-2-thiophenesulfonyl chloride to give **52**, which was then reduced with $LiAlH_4$ to **53** and subsequently acetylated with Ac_2O to give **48**.

Scheme 10 shows the synthetic route by which analogues of **6** possessing different sulfonamide groups were synthesized. Benzonitrile **10a** was reduced with $LiAlH_4$ to give the diamine **54**, which was selectively reacted with 2-acetoxy-isobutyryl chloride at the benzylamine group to give **55**. The latter was reacted with the appropriate arylsulfonyl chloride and then trans-esterified with MeOH in anhydrous potassium carbonate to give the target sulfonamides **56a**–**d**.

RESULTS AND DISCUSSION

All compounds in Table 2 were tested as human CCR4 antagonists in vitro. Antagonist potency was determined by a [^{35}S]-GTPγS radioligand competition functional assay using recombinant CCR4-expressing CHO cell membranes adhered to WGA-coated Leadseeker SPA beads in assay buffer (20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, 0.05% BSA, 40 μg/mL saponin at pH 7.4) with output measured on a Wallac Microbeta Trilux scintillation counter.[30] Another assay using human whole blood was used as a secondary screen to determine potency against the native receptor for the more potent compounds in the primary assay. The assay quantified cytoskeletal reorganization (formation of filamentous (F-) actin) which occurs in a variety of cells in response to chemoattractants and is a prelude to chemotaxis. This was achieved by staining the F-actin with a fluorescent derivative of phalloidin, which binds with high affinity and specificity to the interface between actin monomers in F-actin. The response was measured as an increase in the fluorescence intensity of the target cell population in a flow cytometer and was expressed as a pA_2. In this assay, human $CD4^+$ $CCR4^+$ lymphocytes were identified by staining with antibodies to CD4 and CCR4. Ligand lipophilicity efficiency index (LLE) is a useful metric introduced by Leeson and Springthorpe for assessing the potency and lipophilicity of drug-like molecules during lead optimization.[31] We have used the more recently introduced LLE_{AT}, which combines lipophilicity, potency, and size and

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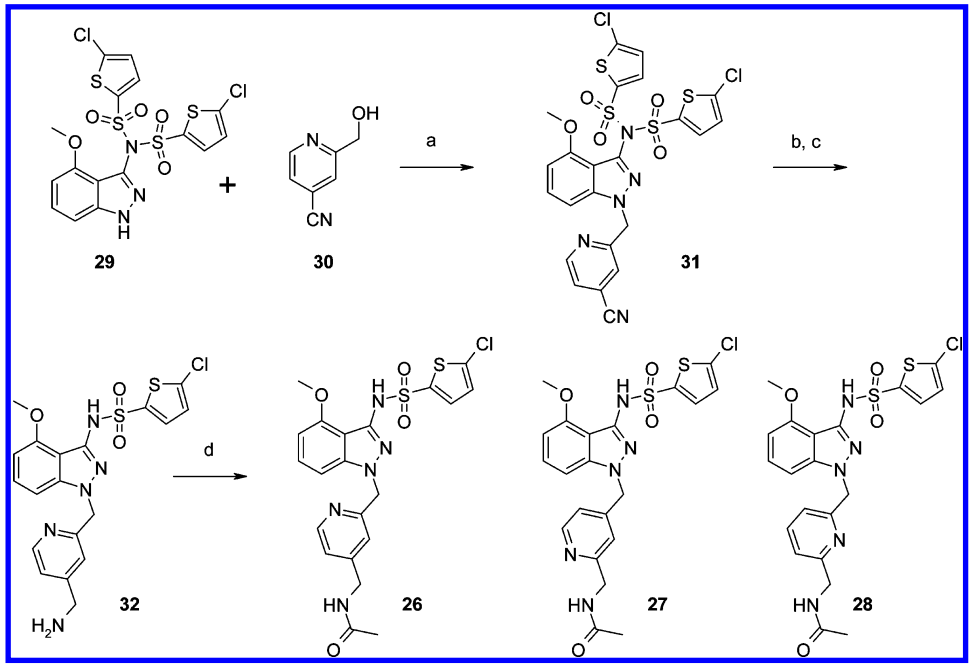
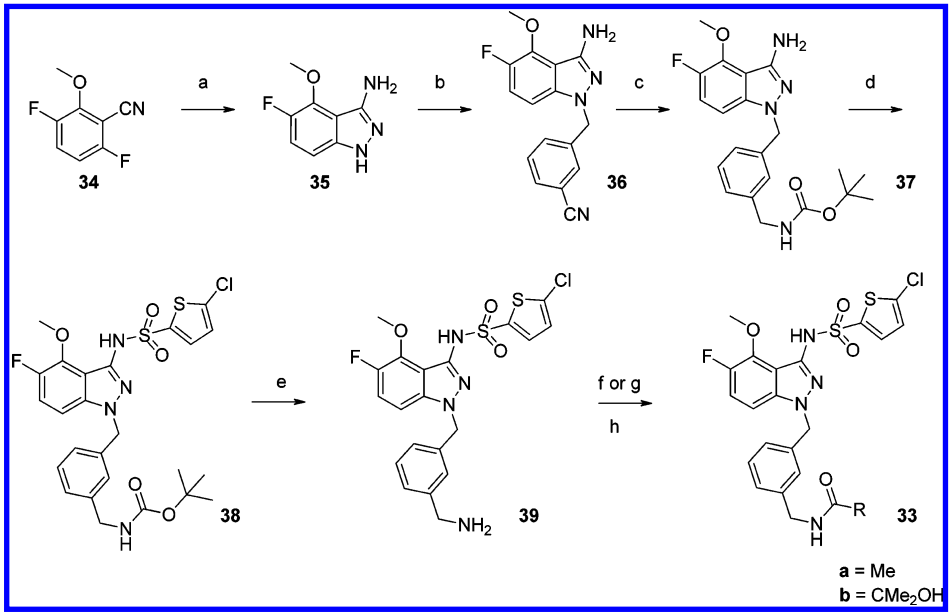
Scheme 6[a]



[a]Reagents and conditions: (a) PPh_3, $^tBuO_2CN{=}NCO_2Bu^t$, THF, 80 °C, 88%; (b) $LiAlH_4$, THF, 1 h; (c) 2 M NaOH, H_2O, MeOH, 70 °C, 53%; (d) AcOH, HATU, Et_3N, DCM, 59%.

Scheme 7[a]



[a]Reagents and conditions (a) $NH_2NH_2{\cdot}H_2O$, EtOH, 80 °C, 25%; (b) 3-chloromethylbenzonitrile, KOH, DMSO, −5 °C, 48%; (c) $NaBH_4$, $NiCl_2$, BOC_2O, THF, MeOH, 65%; (d) 5-chloro-2-thiophenesulfonyl chloride, pyridine, DCM, 55%; (e) TFA, DCM, 45%; (f) (for **33a**) AcOH, HATU, DIPEA, DMF, 27%; (g) (for **33b**) 2-acetoxyisobutyryl chloride, pyridine, DCM; (h) KOH, H_2O, MeOH, 49%.

makes comparisons with conventional ligand efficiency (LE) easier at the commonly used threshold value of 0.3 kcal/mol.[32] In Table 2 the LLE_{AT}, the chromatographic log *D* (chrom log *D* at pH 7.4) and the chemiluminescent nitrogen detection (CLND) kinetic solubility are included for all test compounds in this study.[33] The high throughput CLND solubility assay involves addition of aqueous buffer to a test compound DMSO solution over a period of time until the compound precipitates. Finally, the pK_a of the sulfonamide moiety for selected compounds was measured and presented in Table 2. The test compounds were compared with two standards, compounds **1** and **5**, together with our own sulfonamides **6** and **7a**.[26]

We started our SAR investigations by focusing our attention on C4 substitutions with compounds **7b**−**7k**. The compounds with LLE_{AT} higher than **7a** were the three hydroxyl compounds **7e**, **7g**, and **7i**. All three compounds had higher affinity than **7a**, ranging from 7.7 to 8.2, and lower log *D* by more than a log unit. In addition, the CLND solubilities of **7e** and **7i** were

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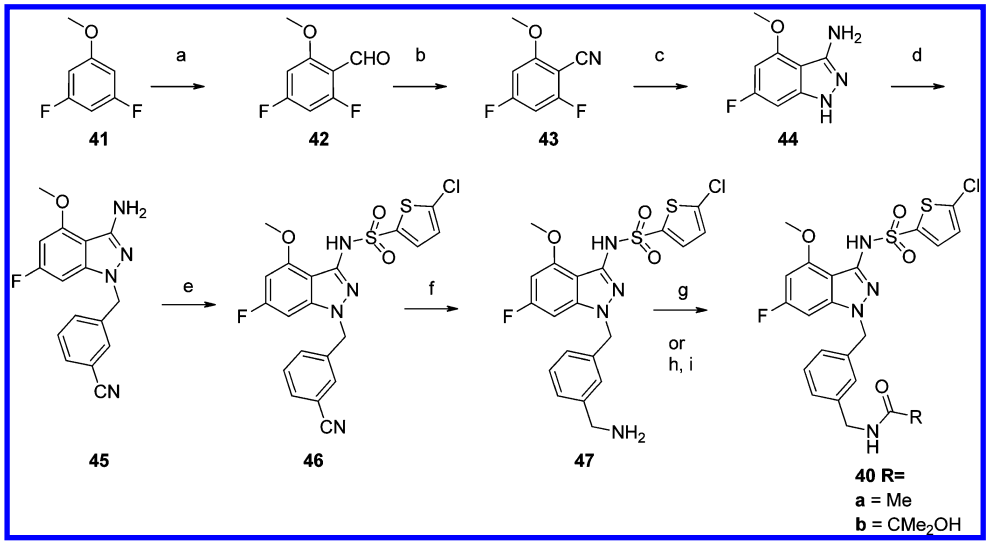
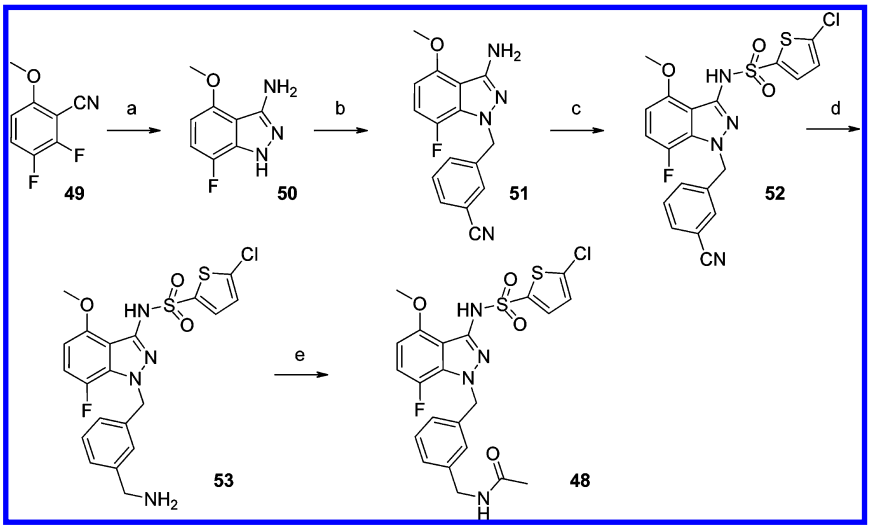
Scheme 8[a]



[a]Reagents and conditions: (a) Cl_2CHOMe, $TiCl_4$, DCM, 57%; (b) NH_2OSO_3H, H_2O, 110 °C, 3 h, 95%; (c) $NH_2NH_2{\cdot}H_2O$, *n*-BuOH, 110 °C, 21%; (d) 3-chloromethylbenzonitrile, KOH, DMSO, 20%; (e) 5-chloro-2-thiophenesulfonyl chloride, pyridine, $CHCl_3$, 45%; (f) $LiAlH_4$, THF, 18%; (g) (for **40a**) Ac_2O, Et_3N, $CHCl_3$, 52%; (h) (for **40b**) 2-acetoxyisobutyryl chloride, pyridine, DCM; (i) K_2CO_3, MeOH, 51%.

Scheme 9[a]



[a]Reagents and conditions. (a) $NH_2NH_2{\cdot}H_2O$, NMP, 150 °C; (b) 3-chloromethylbenzonitrile, KOH, DMSO, 56%; (c) 5-chloro-2-thiophenesulfonyl chloride, pyridine, DCM, 30%; (d) $LiAlH_4$, THF, 36%; (e) Ac_2O, Et_3N, DCM, 34%.

higher than **7a**. The higher affinity did not translate to the whole blood actin polymerization assay but remained at approximately the same level as **7a**, with the highest potency of 6.3 for alcohol **7i**. On the basis of these data, compounds **7e**, **7g**, and **7i** were selected for further investigation. It was interesting to note that both the AZ and Ono compounds, **4** and **5**, possessed an *ortho*-alkoxy substituent capable of hydrogen bonding with the sulfonamide NH forming a five-membered ring. Our own candidate **6** had a C4 methoxy substituent capable of hydrogen bonding with the sulfonamide NH, forming a six-membered ring. The effect of hydrogen bonding to the acidic sulfonamide NH would be to alter its acidity, leading us to hypothesize that there might be a relationship between acidity and potency. The effects of ortho substituents in the dissociation of substituted *N*-phenyl benzenesulfonamides were reported by Ludwig, who demonstrated that the pK_a increased in the case of an *ortho*-methoxy group in the aniline ring.[34] The pK_a of all the C4 substituted analogues (**7a**–**k**) was measured and is shown in Table 2. Comparing **7a** with **7b** the MeO- group increased the pK_a in line with Ludwig's observations. Despite measurable differences in the acidity of the various analogues' sulfonamide NH, there was no correlation between sulfonamide pK_a and GTPγS affinity. Interestingly, the affinity of the two analogues that cannot form a hydrogen bond, namely the *des*-methoxy analogue **7b** and the cyano analogue **7f**, have the lowest affinity (6.6 and 6.5, respectively), whereas the three hydroxyl analogues **7e**, **7g**, and **7i**, which are capable of hydrogen bonding, have the highest affinity (7.9, 8.2, and 7.7, respectively). We have therefore hypothesized that the effect of hydrogen-bonding would be to hold the analogues in a locked active conformation. In the absence of suitable crystals

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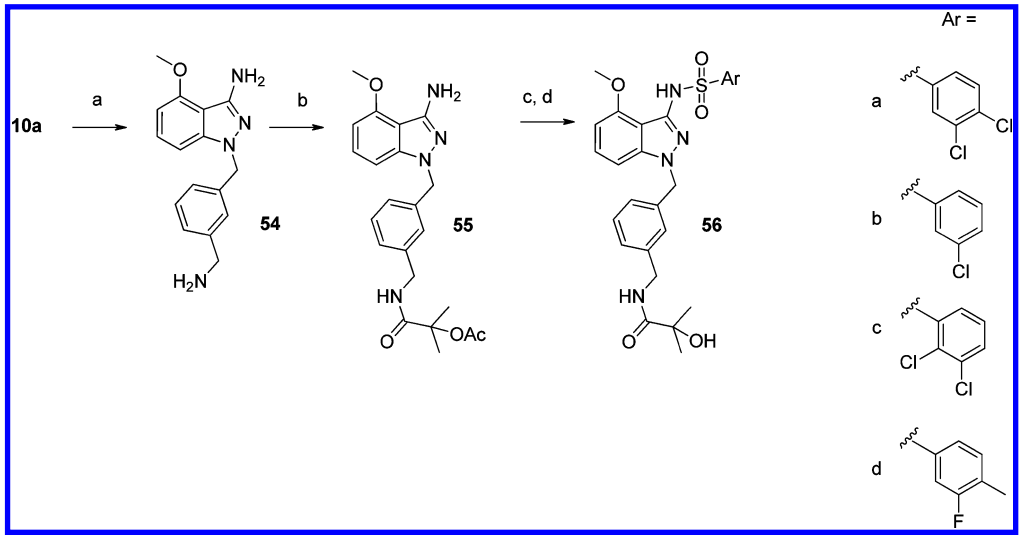
Scheme 10[a]



[a]Reagents and conditions: (a) $LiAlH_4$, THF, 50%; (b) 2-acetoxy-isobutyryl chloride, Et_3N, DCM, 87%; (c) arylsulfonyl chloride, pyridine, DCM; (d) K_2CO_3, MeOH, 39−55%.

of **6** for an X-ray diffraction study, we have examined smaller fragments of **6** such as the *N*1-methyl analogues **57** and **58**. Fragment **57** gave suitable crystals for a small-molecule X-ray diffraction study (Figure 2). In this structure, an intramolecular hydrogen bond between the sulfonamide and the C4-OMe group is clearly observed [N−H, 0.80(3) Å; H···O, 2.52(3) Å; N···O, 3.036(3) Å; and ∠N−H···O, 124(3)°]. A consequence of this hydrogen bond is to allow the plane normals to the thiophene ring and the indazole core to be approximately orthogonal [83.73(7)°]. Fragment **58** possesses a 3-thiophene substituent at C4, which removes the possibility of this intramolecular hydrogen bond. Figure 3 shows that the above two plane normals are now closer to being parallel [17.2(2)°]. A search of the Cambridge Structural Database (v 5.33, Nov 2011) for [5,6] bicyclic cores containing any combination of carbon, nitrogen, and oxygen atoms, and being substituted with a sulfonamide moiety and an oxygen atom at the equivalent 3- and 4-position of the indazole ring system, yielded no hits, confirming the novel arrangement reported herein. Moreover, the GTPγS affinity of fragment **57** was 6.4, whereas that of **58** was 5.8. Replacement of the *N*1-Me substituent of **57** with a benzyl group leads to analogues with increased potency, whereas in the case of **58** lead to nonadditive effects. These data are consistent with the theory that disruption of the intramolecular hydrogen bond leads to an alternative conformation and a different binding mode, which in turn leads to reduced potency.

Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius. The intramolecular hydrogen bond is indicated by a dashed line.

Anisotropic atomic displacement ellipsoids for the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are displayed with an arbitrarily small radius.

In our preliminary communication, we have reported that amide and hydroxyl-amide groups at the N1 benzyl group increased both the potency and solubility.[26] Recently, oxetanes were reported as groups that increase the solubility of compounds,[35] therefore, oxetane amides **24a** and **24b**, and related cyclic ether amides **24c** and **24d**, were investigated. The LLE_{AT} of all four compounds was inferior to **7a**, whereas the chrom log *D* was similar or even higher than **7a**. Interestingly, the CLND solubility of **24a**, **24c**, and **24d** was slightly increased compared to **7a**, whereas **24b** was similar. In comparing the 3-oxetane with the 2-oxetane analogues (**24a** vs **24b**), the former had higher solubility and lower chrom log *D*, which might be due to the greater exposure of the oxygen atom.

Another way of increasing the solubility would be the introduction of an ionizable group such as an amino group. All three amines, **24e**, **24f**, and **24g**, had identical affinity, lower log *D*, and slightly increased CLND solubility. Furthermore, all three analogues had high whole blood potency, and indeed they were the most potent compounds in this assay, making them suitable for further investigation. It was interesting to investigate the reason that these amines were uniformly more potent in the whole blood assay, and we hypothesized that this might be due to lower plasma protein binding. The rapid equilibrium dialysis human plasma protein binding for a range of analogues is presented in Table 3. The three strongly basic analogues **24e**, **24f**, and **24g** had the lowest plasma protein binding, indicating that the higher potency in the whole blood actin polymerization assay was related to the higher levels of unbound compound. The four less basic analogues **24h−k** containing a morpholine moiety had very high affinity in the GTPγS assay. Compounds **24j** and **24k**, which are enantiomers, were found to have identical activity with a pIC_{50} value of 8.2, one of the highest values in this series. However, due to their high molecular weight, their LLE_{AT} value was slightly reduced at 0.26. The solubility of analogues **24h−k** was higher as expected, making these compounds worthy of further investigation. The basic analogue containing the 3-oxetane **24l** was less potent with a LLE_{AT} of 0.2 and low solubility, and it was therefore rejected.

Another approach to increase the solubility of analogues of **7a** was replacement of the phenyl ring of the benzylic substituent by a heterocyclic ring, such as pyridine, in analogues **26−28**. These analogues had reduced lipophilicity and very good LLE_{AT}, however, their potency was lower than **7a** and they were not examined any further. Similarly introduction of

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Table 2. In Vitro Data pIC_{50} for GTPγS Binding Assay, Human Whole Blood F-Actin Polymerization pA_2, Ligand Efficiency, Chrom log *D*, CLND Solubility, and pK_a

compd	GTPγS pIC_{50}	$LLE_{(AT)}$	chrom log *D*	CLND[a] solubility (μg/mL)	hWB actin polymerization pA_2 (*n*)	pK_a
1	8.26 ± 0.01 (150)	0.25	2.9	≥145	7.3 ± 0.3 (4)	ND
5	8.41 ± 0.02 (147)	0.27	2.9	193	6.6 ± 0.1 (24)	ND
6	7.83 ± 0.02 (113)	0.27	4.3	≥162	6.2 ± 0.1 (11)	6.54
7a	7.50 ± 0.04 (10)	0.28	4.1	≥124	6.0 ± 0.1 (7)	6.60
7b	6.6 ± 0.0 (4)	0.24	3.3	≥117	ND	5.93
7c	7.5 ± 0.1 (2)	0.25	3.8	≥226	5.2 ± 0.2 (2)	6.03
7d	7.1 ± 0.1 (2)	0.26	3.0	≥139	5.3 ± 0.2 (2)	5.62
7e	7.9 ± 0.2 (2)	0.32	2.9	≥166	6.1 ± 0.1 (2)	6.12
7f	6.5 ± 0.1 (4)	0.26	2.5	≥155	ND	5.40
7g	8.2 ± 0.1 (2)	0.35	2.6	≥113	6.0 ± 0.3 (2)	5.66
7h	7.0 ± 0.1 (2)	0.27	4.8	57	ND	ND
7i	7.7 ± 0.1 (4)	0.31	2.7	≥208	6.3 ± 0.1 (3)	5.62
7j	7.0 ± 0.1 (2)	0.26	2.9	≥159	ND	5.46
7k	7.0 ± 0.1 (2)	0.24	3.7	≥188	5.1 ± 0.1 (2)	5.60
24a	7.7 ± 0.2 (4)	0.25	4.0	≥173	6.0 ± 0.2 (3)	ND
24b	7.5 ± 0.1 (4)	0.27	4.5	124	ND	ND
24c	7.4 ± 0.2 (4)	0.23	4.9	≥153	6.1 ± 0.1 (2)	ND
24d	7.5 ± 0.2 (4)	0.26	4.4	≥193	6.3 ± 0.1 (3)	ND
24e	7.8 ± 0.1 (2)	0.27	3.1	191	6.7 ± 0.0 (5)	ND
24f	7.8 ± 0.2 (6)	0.28	3.3	≥180	6.85 ± 0.04 (4)	ND
24g	7.8 ± 0.1 (10)	0.24	3.3	≥220	6.9 ± 0.1 (4)	ND
24h	7.6 ± 0.2 (4)	0.27	3.9	≥250	6.25 ± 0.15 (4)	ND
24i	7.8 ± 0.1 (6)	0.28	3.9	≥212	7.1 ± 0.3 (4)	ND
24j	8.2 ± 0.1 (3)	0.26	4.4	≥203	6.6 ± 0.2 (6)	ND
24k	8.2 ± 0.0 (3)	0.26	4.4	≥180	6.6 ± 0.2 (6)	ND
24l	6.6 ± 0.2 (4)	0.20	4.3	122	ND	ND
26	6.8 ± 0.1 (4)	0.32	2.6	≥141	<5.0 (1)	ND
27	7.0 ± 0.1 (6)	0.33	2.5	≥161	ND	ND
28	6.9 ± 0.2 (2)	0.32	3.0	≥182	ND	ND
33a	7.9 ± 0.1 (3)	0.29	4.1	≥199	5.5 ± 0.2 (3)	ND
33b	7.4 ± 0.1 (2)	0.25	4.1	≥174	6.2 ± 0.0 (2)	ND
40a	7.4 ± 0.2 (4)	0.26	4.1	≥161	6.0 ± 0.2 (5)	ND
40b	7.8 ± 0.0 (14)	0.26	4.2	≥198	6.2 ± 0.1 (4)	ND
48	7.1 ± 0.1 (4)	0.25	4.0	≥177	ND	ND
56a	6.4 ± 0.3 (4)	0.19	5.1	76	ND	6.76
56b	7.3 ± 0.2 (4)	0.24	4.5	≥198	ND	7.06
56c	6.4 ± 0.2 (4)	0.19	4.7	157	ND	6.82
56d	7.8 ± 0.2 (3)	0.25	4.5	36	5.9 ± 0.2 (3)	7.26

[a]CLND solubility values that are within 85% of maximum possible concentration (as determined from DMSO stock concentration) are reported as ≥. ND: not determined. When $n < 3$, SEM is the SD.

nitrogen in the indazole benzene ring to provide aza-indazole analogues gave compounds with reduced potency (data not shown).

Substitution in the aromatic ring of the indazole core at C5, C6, and C7 was not tolerated, apart from very small groups. Of particular interest was substitution with fluorine, as this might reduce metabolism of the phenyl ring. The C5 fluoro analogue **33a** was more potent than **7a**, however, it had a lower whole blood potency, whereas analogue **33b** was less potent in the GTPγS assay than **6** but equipotent in the whole blood assay. Since neither compound had any additional advantage over **7a** nor **6**, these analogues were not progressed any further. Substitution with fluorine at C6 gave **40a** and **40b**. Both compounds were equipotent in both assays with their respective analogues **7a** and **6**. The LLE_{AT} was slightly reduced as a result of the higher molecular weight, and the CLND solubility for both **40a** and **40b** was marginally improved. Compound **40b** was chosen for further investigation. The C7 fluoro analogue of **7a**, **48**, was of lower potency and lower solubility and was rejected.

Finally, variation of the sulfonamide moiety of **6** was investigated with a large number of analogues, including alkyl, cycloalkyl, heterocyclic, heteroaryl, and aryl analogues; four of the latter are shown in Table 2. None of these analogues however demonstrated any improvement over compound **6**.

The passive membrane permeability (P_{ex}) of the more potent compounds across Madin–Darby canine kidney–multidrug resistance 1 (MDCKII-MDR1) cells in the presence of a potent P-glycoprotein inhibitor is shown in Table 3. The permeability of **6** and its 6-fluoro analogue **40b** was good but lower than **7a**, and this might be a reflection of the additional hydrogen bond donor in these two analogues. The compound with the highest cell membrane permeability was the morpholine **24k**, which was less basic and did not have any additional hydrogen bond donors, whereas the morpholine **24i** with an additional hydrogen bond donor was lower than **24k** but still at the

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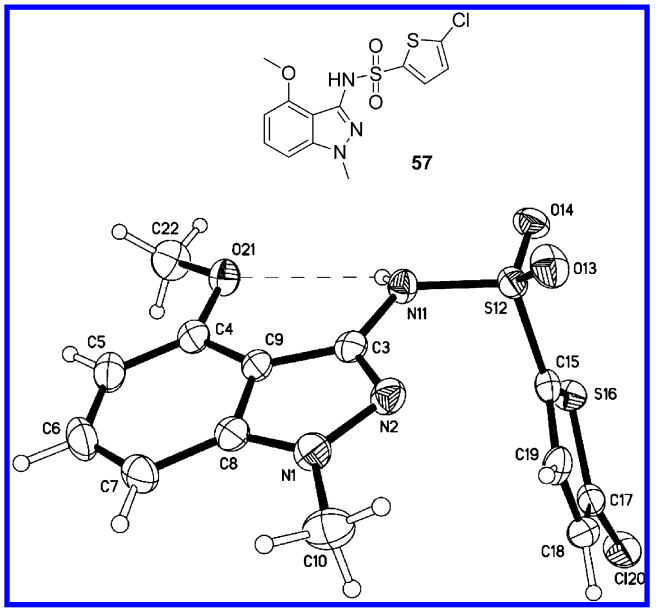

Figure 2. The 2D connectivity and 3D conformation taken from the crystal structure of **57**.

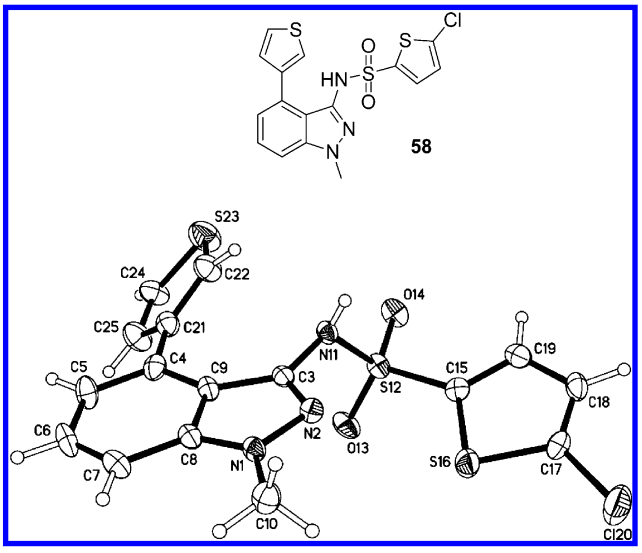

Figure 3. The 2D connectivity and 3D conformation taken from the crystal structure of **58**.

same levels as **6** and **40b**. The compounds with the lowest permeability were **7i**, **24e**, and **24g**, which had extra hydrogen bond donors and/or were strongly basic. The only odd compound with good permeability but being strongly basic and possessing two additional hydrogen bond donors was **24f**, the amino analogue of **6**. It is interesting to speculate whether this was due to an active transport mechanism; however, we have no evidence for this. The compounds shown in Table 3 were progressed to preliminary CD rat pharmacokinetic studies in vivo and are compared with the previously reported data for compounds **7a** and **6**.[26] Each compound was dosed iv and po at 1 mg/kg, $n = 2$ for each leg of the study, except for the iv leg of **24g**, which had $n = 1$. The compounds were dosed as solutions in DMSO−PEG200−H_2O (5:45:50), except for **24k**, which was dosed in the ratio of 10:40:50. The bioavailability of the phenolic analogue **7e** was 12%, whereas that of the secondary alcohol **7i** was 35%, which was greatly diminished by comparison with **7a**, and consequently their progression was terminated. The strongly basic analogues **24e** and **24g** had low bioavailability, whereas the less basic morpholines **24i** and **24k** had bioavailability of 32% and 68%, respectively. Compound **24f**, the strongly basic amino analogue of **6**, also had reduced oral bioavailability compared to **6** although it was slightly higher at 29% than the other strongly basic compounds, which may be a reflection of its higher permeability, and further highlighting the possibility of an active transport mechanism for this analogue. The morpholines were considered worthy of further investigation in the dog. The 6-fluoro analogue **40b** had high bioavailability similar to that of **6**. The five compounds shown in Table 4 were progressed to pharmacokinetic studies in the beagle dog and are compared with the previously reported data for compounds **7a** and **6**.[26] Each compound was dissolved in DMSO−PEG200−H_2O (5:45:50) and dosed iv at 0.5 mg/kg, with $n = 2$ per compound, except for **24k** ($n = 1$). Two compounds, **24e** and **24i**, were progressed to the po leg of the study at a dose of 1 mg/kg, $n = 2$ and had bioavailability of 81% and 39%, respectively. The clearance of both **24e** and **24i** was much higher than that of **6** and were therefore not progressed. The remaining three compounds, **24f**, **24g**, and **24k**, had at least 2-fold higher clearance than **6** and were therefore of no further interest. The only remaining compounds were **6** and **40b**. Both compounds had a very similar rat pharmacokinetic profile and very similar CLND solubility. The equilibrium solubility of **6** and **40b** in a range of physiologically relevant media was measured using HPLC quantification and is

Table 4. Beagle Dog Pharmacokinetic Parameters

	24e	24f	24g	24i	24k	7a	6
Cl (mL/min/kg)	17	18	32	41	22	18	9
Vdss (L/kg)	6	5.0	11	3.0	5.7	3.2	2.2
$T_{1/2}$ (h)	5.9	2.4	0.95	2.6	2.6	1.6	2.6
F%	81	NA[a]	NA	39	NA	49	97

[a]NA: not applicable.

Table 3. Human Plasma Protein Binding (PPB) Measured by Equilibrium Dialysis, Membrane Permeability (P_{ex}) across MDCK Cells, and CD Rat Pharmacokinetic Parameters

	7e	7i	24e	24f	24g	24i	24k	40b	7a	6
human PPB (%)	100	ND[a]	94.6	96	92.2	98.2	98.6	ND	99.5	99
P_{ex} (nm/s)	ND	27.5	50	121	45	97	300	111	254	132
Cl (mL/min/kg)	19	13	17	15	29	18	24	19	5	15
Vdss (L/kg)	1.2	3.5	0.79	0.84	2.6	0.78	1	1.5	0.57	1.9
iv $T_{1/2}$ (h)	1.2	3.7	0.8	1.3	3	1.2	1.3	1.8	1.6	2.5
F%	12	35	19	29	<1	32	68	90	100	85

[a]ND not determined.

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summarized in Table 5. The solubility of both compounds was found to be low in all physiologically relevant media such as simulated gastric fluid (SGF), water, fed-state simulated intestinal fluid (FeSSIF), and fasted-state simulated intestinal fluid (FaSSIF). The 24 h water equilibrium solubility for **6** was 57 μg/mL, whereas for **40b** was 32 μg/mL. These figures were substantially lower than the values obtained from the high throughput CLND kinetic solubility measurement of 162 and 198 μg/mL, respectively (Table 2). The difference in kinetic vs equilibrium solubility of **6** obtained from either solid material or from DMSO stock solutions was investigated using NMR quantification. Solubility from solid in pH 7.4 phosphate buffered saline (PBS) confirmed the data from the HPLC quantified measurements, with the solubility increasing with time. Kinetic solubility from 10 mM DMSO stock solution by adding pH 7.4 buffer to give a 5% DMSO final concentration (as with the CLND determination) provided a solubility of about 270 μg/mL. This solution was stable even after 100 h. Solubility from solid in the presence of a small amount of MeOH (10 μL) to break up initial crystal lattice showed a marked difference to the solubility obtained from solid alone. The compound freely dissolved in MeOH and when diluted down with PBS yielded an initial solution in excess of 270 μg/mL. This solution, unlike the DMSO solution, was not stable; the solubility decreased with time until equilibrium was reached at the same final concentration as that obtained from solid alone. These data are consistent with the formation of very stable supersaturated solution of **6** in the case of the high throughput CLND solubility assay, giving rise to the apparent higher solubility figures. The stability of **6** in the solid state was examined for 1 month at 50 °C, and at 40 °C−75% relative humidity. No changes in the main peak, impurities, or physical properties were observed, confirming that **6** was stable in the solid state.

Table 5. Equilibrium Solubility after 24 h of 6 and 40b in Various Physiologically Relevant Media and pHs[a]

media	**6** (μg/mL)	**40b** (μg/mL)
water	57	32
simulated gastric fluid (pH 1.6)	1	1
FeSSIF (pH 6.5)[b]	23	33
FaSSIF (pH 6.5)[c]	10	36
saline	73	ND[e]
pH 2[d]	3	ND
pH 7[d]	10	ND
pH 8[d]	56	ND
pH 10[d]	1324	ND

[a]Compounds were quantified by HPLC. [b]FeSSIF = fed state simulated intestinal fluid. [c]FaSSIF = fasted state simulated intestinal fluid. [d]Britton−Robinson buffer was used for the pH 2−10 determinations. [e]ND: not determined.

On the basis of the interesting solubility findings of **6**, we speculated whether there was any difference between the rat bioavailability of **6** following administration as a solution in DMSO−PEG200−H_2O (5:45:50) (F = 85% at 1 mg/kg, Table 3) and following administration of a suspension of wet bead milled material (particle size <1 μm). Following administration of the suspension at 1, 10, and 30 mg/kg to the rat, the oral exposure of **6** increased with increasing dose and bioavailability remained high (53%, 80%, and 101%, respectively). While the interanimal variability was low following the 10 and 30 mg/kg dose, variability was high following the 1 mg/kg dose (range of 26−72%). These findings gave us confidence that despite the low solubility of **6** high bioavailability could be achieved from a solid dose and encouraged further progression of **6**.

The selectivity of **6** for CCR4 over other chemokine receptors was evaluated in a series of GTPγS binding assays at Euroscreen. Compound **6** had a pIC_{50} of 7.4 for CCR4, and the pIC_{50} for CCR1, CCR2, CCR8, CCR10, CXCR1, CXCR2, CXCR3, and CXCR4 was <5, whereas for CCR5 was 5.2. The selectivity of **6** against a panel of 7TM receptors, ion channels, enzymes, transporters, and nuclear receptors was evaluated in agonist or antagonist mode as appropriate. The only off-target activity of note was weak antagonism (pXC_{50} 5.1) at the α1 nicotinic ACh receptor, which potentially leads to neuromuscular blockade and generalized muscle relaxation. At this level of activity, any physiological effect is unlikely. There was also moderate inhibitory activity at the organic anion transporting polypeptide OATP1B1 transporter (PXC_{50} 6.4). OATPs form a superfamily of sodium-independent transport systems that mediate the trans-membrane transport of a wide range of amphipathic endogenous and exogenous organic compounds. OATPs are involved in the uptake of organic anions from the blood to the liver and therefore may be involved in drug distribution, metabolism, and elimination. Compound **6** was negative in the Ames test and not toxic to membranes and mitochondria in the HepG2 Cell Health assay. Furthermore, there were no cardiovascular issues associated with **6** in rabbit ventricular wedge (up to 10 μM), no cardiac repolarization, conduction, contractility, nor any liability for torsadogenic (TdP) arrhythmias. Similarly in the conscious rat, there were no treatment related effects on body temperature, cardiovascular, or electrocardiographic parameters at 30, 100, and 300 mg/kg po. In addition, no toxic effects were observed in a 7-day repeat oral study in the rat.[36]

Compound **6** was very highly bound (99% or greater) to plasma proteins in rat, dog, and human plasma. The extent of binding was determined in CD rat, beagle dog, and human plasma using rapid equilibrium dialysis at a nominal concentration of 1 μg/mL (n = 3). The modest half-life in rat and dog are consistent with the low/moderate Cl and low/moderate volume of distribution, suggesting that the majority of compound was removed effectively from the body and that the plasma protein binding does not appear to be restrictive either for Cl or Vd. The in vitro clearance of **6** was very low in three species: rat, dog, and human. There was no evidence of tissue accumulation or toxicity on repeat oral administration in rat or dog. This reinforces the lack of restrictive binding in vivo, although it may also indicate that there is a nonmetabolic clearance involved. It is possible that there was an underlying longer terminal phase that was not detected due to being below the limit of detection of the blood assay.

Because of the differences in immunology between animals and human, the complexity of chemokine systems across species, the presence of multiple chemokine ligands, and the uncertain relevance of animal models to clinical disease, no in vivo anti-inflammatory data has been generated for **6**. Therefore a microdose study administering radiolabeled **6** in man was undertaken to provide preliminary information on the distribution and clearance in man and hence allow accurate modeling and dose predictions for the oral first-time-in-human study.[36] C3-[^{14}C]-labeled **6** was synthesized by the same route as **6** starting from [^{14}CN]-**8**. The microdose study was conducted in healthy male volunteers receiving an intravenous

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infusion of 100 μg [^{14}C]-**6** per volunteer. The derived human pharmacokinetic parameters confirmed that the compound had a low to moderate clearance. These data, together with absorption data from the rat and dog PK studies, were used in simulations to predict blood exposure in man.[36]

As mentioned in our Introduction, the AstraZeneca group have reported in 2008 the presence of an intracellular allosteric site on CCR4 and CCR5 receptors to which small pyrazine sulfonamides bind.[27] Further pharmacological studies were performed by our group to determine which binding site on CCR4 mediated the effects of the indazole sulfonamide series. These experiments used a combination of functional antagonist interaction studies in isolated T cell actin polymerization assays and radioligand binding with site-selective tritiated antagonists **1** and **4**. The studies indicated that there are three distinct binding sites on the CCR4 receptor, the orthosteric site to which the macromolecular ligands MDC and TARC bind and two additional allosteric sites to which small molecules bind. Lipophilic amines such as compound **1** and **2** bind to an allosteric site denoted arbitrarily as site I. Compound **6** and its analogues bind to site II, the allosteric modulatory site, which is accessible from the cytoplasmic face of the receptor and which may also exist in other chemokine receptors.[37] Site II overlaps with the binding site for the pyrazine sulfonamides **4** and **5**. These experiments will be the subject of a future publication.[38] The presence of multiple antagonist binding sites on CCR4, at least one of which is allosteric, opens the potential for the design of molecules which have selective effects on its two chemokine agonists. Also, compounds which bind to more than one of these sites would be predicted to have greater inhibitory activity than the current site-selective compounds.

CONCLUSION

A novel series of indazole arylsulfonamides were synthesized and examined as allosteric human CC chemokine receptor 4 antagonists. The preferred C4 substituents were methoxy- or hydroxy-containing groups. Fluoro-groups at C5, C6, or C7 were tolerated, with the C6 analogues being preferred. The most potent *N*3-substituent was 5-chlorothiophene-2-sulfonamide. A variety of potent *N*1 *meta*-substituted benzyl groups were identified. The most potent substituents contained a 3-methylaminoamide group possessing an α-amino moiety. Strongly basic amino groups had low oral bioavailability in rat and/or dog pharmacokinetic studies. Less basic analogues, such as morpholines, had better apparent permeability and moderate/high bioavailability in rat, however, they tended to have high clearance in dog. The most potent compound with high bioavailability in both rat and dog was the tertiary-hydroxy analogue **6** (GSK2239633A). This compound was selective against other chemokine receptors and a panel of 7TM receptors, ion channels, enzymes, transporters, and nuclear receptors. Compound **6** had high permeability in MDCK cells and was highly bound to plasma proteins. It had low solubility in all physiologically relevant media, with the solubility increasing dramatically at pH 10. This suggested that **6** might have low oral bioavailability at higher doses. However, the oral exposure of **6** in the rat, following administration of a suspension of wet bead milled material, increased with increasing dose up to 30 mg/kg. Compound **6** was selected for further development, and the clinical studies will be reported shortly.[36]

EXPERIMENTAL SECTION

Organic solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F_{254} plates. Products were visualized under UV light and/or by staining with aqueous $KMnO_4$ solution. LCMS analysis was conducted on either System A, an Acquity UPLC BEH C18 column (2.1 mm × 50 mm ID, 1.7 μm packing diameter) eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using the following elution gradient 0.0−1.5 min 3−100% B, 1.5−1.9 min 100% B, 1.9−2.0 min 3% B, at a flow rate of 1 mL min^{-1} at 40 °C. The UV detection was an averaged signal from wavelength of 210 to 350 nm, and mass spectra were recorded on a mass spectrometer using alternate-scan electrospray positive and negative mode ionization (ES +ve and ES −ve); or System B Sunfire C_{18} column (30 mm × 4.6 mm ID, 3.5 μm packing diameter) eluting with 0.1% formic acid in water (solvent A), and 0.1% formic acid in MeCN (solvent B), using the following elution gradient 0−0.1 min 3% B, 0.1−4.2 min 100%, 4.2−4.9 min 3% B, 4.9−5.0 min 3% B at a flow rate of 3 mL min^{-1} at 30 °C; or System C Acquity UPLC BEH C_{18} column (50 mm × 2.1 mm ID, 1.7 μm packing diameter) eluting with 10 mM ammonium bicarbonate in water adjusted to pH10 with ammonia solution (solvent A) and MeCN (solvent B) using the following elution gradient 0−1.5 min 1−97% B, 1.5−1.9 min 97% B, 1.9−2.0 min 100% B at a flow rate of 1 mL min^{-1} at 40 °C. Column chromatography was performed on Flashmaster II. The Flashmaster II is an automated multiuser flash chromatography system, available from Argonaut Technologies Ltd, which utilizes disposable, normal phase, SPE cartridges (2−100 g). Mass-directed autopreparative HPLC (MDAP) was conducted on a Sunfire C18 column (150 mm × 30 mm ID, 5 μm packing diameter) at ambient temperature eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using an appropriate elution gradient over 15 min at a flow rate of 40 mL min^{-1} and detecting at 210−350 nm at room temperature. Mass spectra were recorded on Micromass ZMD mass spectrometer using electro spray positive and negative mode, alternate scans. The software used was *MassLynx* 3.5 with *OpenLynx* and *FractionLynx* options. ^{1}H NMR spectra were recorded at 400 MHz unless otherwise stated. The chemical shifts are expressed in ppm relative to tetramethylsilane. High resolution positive ion mass spectra were acquired on a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer. Optical rotations were measured with an Optical Activity AA100 digital polarimeter. Analytical chiral HPLC was conducted on Chiralpak column (250 mm × 4.6 mm) eluting with an appropriate ratio of EtOH−heptane for 30 min at room temperature, flow rate 1 mL min^{-1}, injection volume 15 μL, detecting at 215 nm. The purity of all compounds screened in the biological assays was examined by LCMS analysis and was found to be ≥95% unless otherwise specified. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

4-(Methyloxy)-1*H*-indazol-3-amine (9a). A mixture of 2-fluoro-6-(methyloxy)benzonitrile (**8a**) (10 g, 66 mmol) and hydrazine hydrate (9.63 mL, 198 mmol) in *n*-butanol (100 mL) was heated to reflux under nitrogen for 18 h. The reaction mixture was allowed to cool, water (300 mL) was added, and the organic phase was removed. The solid in the aqueous phase was collected by filtration and dried in vacuo at 40 °C to give a white solid (0.6g). The butanol phase was evaporated in vacuo, and the residue and the aqueous mother liquors were combined and extracted using ethyl acetate (2 × 200 mL). The combined ethyl acetate extractions were dried ($MgSO_4$) and evaporated in vacuo. The residue was dissolved in DCM and applied to a 100 g silica cartridge. This was eluted with cyclohexane (500 mL), cyclohexane−ethyl acetate (1:1, 500 mL), and ethyl acetate (500 mL). The required fractions were combined and evaporated in vacuo to give **9a** (9.92 g, 92%) as an off-white solid. MS ES + ve *m*/*z* 164 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 11.35 (1H, s), 7.10 (1H, t, *J* = 8 Hz), 6.76 (1H, d, *J* = 8 Hz), 6.29 (1H, d, *J* = 8 Hz), 4.95 (2H, br s), 3.85 (3H, s).

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3-{[3-Amino-4-(methyloxy)-1*H*-indazol-1-yl]methyl}-benzonitrile (10a). A solution of ground potassium hydroxide (6.75 g, 120 mmol) in DMSO (300 mL) was treated with **9a** (7.85 g, 48.1 mmol) at room temperature under nitrogen, and this gave a deep-red solution. After 5 min, the red solution was treated with 3-cyanobenzyl chloride (8.84 g, 58.3 mmol) in one portion. The reaction mixture was stirred for 20 min and then poured into water (500 mL), forming an emulsion. The emulsion was extracted with chloroform (3 × 500 mL). The combined organic solutions were washed with water (400 mL) and passed through a hydrophobic frit. The solvent was removed in vacuo, and the residue was applied to a 340 g silica cartridge and eluted with a gradient of 0−100% ethyl acetate in cyclohexane over 8 column volumes (CV). The solvent was evaporated under reduced pressure to give an orange solid, which was triturated in a mixture of ethyl acetate (10 mL) and cyclohexane (90 mL). The solid was collected by filtration and washed with cyclohexane (50 mL). The solid was dried in vacuo to give **10a** (7.89 g, 59%) as a pale-orange solid: MS ES +ve *m/z* 279 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.61−7.56 (1H, m), 7.49−7.43 (3H, m), 7.27 (1H, t, *J* = 8 Hz), 6.76 (1H, d, *J* = 8 Hz), 6.40 (1H, d, *J* = 8 Hz), 5.36 (2H, s), 5.00−4.10 (2H, br), 4.00 (3H, s).

5-Chloro-*N*-[1-[(3-cyanophenyl)methyl]-4-(methyloxy)-1*H*-indazol-3-yl]-2-thiophenesulfonamide (11a). To **10a** (7.89 g, 28.3 mmol) was added a solution of 5-chloro-2-thiophenesulfonyl chloride (6.15 g, 28.3 mmol) in pyridine (9.17 mL, 113 mmol) under nitrogen at room temperature. An exothermic reaction occurred which went deep-red colored. After 40 min, the reaction mixture was partitioned between ethyl acetate (500 mL) and 2 M hydrochloric acid (500 mL). The aqueous phase was extracted with ethyl acetate (400 mL). The combined organic solutions were dried ($MgSO_4$) and evaporated in vacuo. The deep-red residue was dissolved in DCM and applied to a 340 g silica cartridge. The cartridge was eluted with a gradient of 0−10% ethyl acetate in dichloromethane over 8 CV. Evaporation of the appropriate fractions gave **11a** (11.1 g, 85%) as an off-white solid: MS ES +ve *m/z* 459/461 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.75 (1H, br s),7.60−7.56 (1H, m), 7.51 (1H, d, *J* = 4 Hz), 7.44−7.39 (2H, m), 7.35 (1H, br s), 7.27 (1H, t, *J* = 8 Hz), 6.82 (1H, d, *J* = 4 Hz), 6.80 (1H, d, *J* = 8 Hz), 6.42 (1H, d, *J* = 8 Hz), 5.47 (2H, s), 3.95 (3H, s).

***N*-[1-{[3-(Aminomethyl)phenyl]methyl}-4-(methyloxy)-1*H*-indazol-3-yl]-5-chloro-2-thiophenesulfonamide hydrochloride (12a).** A lithium aluminum hydride solution in ether (1M, 60.5 mL) was added slowly under nitrogen to a cooled solution of **11a** (11.1 g, 24.2 mmol) in THF (150 mL), maintaining temperature below 10 °C. The suspension was stirred at room temperature for 1 h and then was quenched by addition of water (7 mL), followed by a 2 M solution of sodium hydroxide (42.5 mL). After stirring for 30 min, the solid was removed by filtration and washed with THF. The combined filtrate and washings were loaded on two 70 g SCX-2 cartridges. The cartridges were washed with methanol (1 L) and then eluted with 10% 2 M hydrochloric acid in methanol (2 L). The required fractions were combined and concentrated under reduced pressure. The resultant solid was collected by filtration and washed with water. The solid was dried in vacuo to give the hydrochloride salt of **12a** (9.3 g, 77%) as a white solid: MS ES +ve *m/z* 463/465 $(M + H)^+$. ^{1}H NMR δ (DMSO-d_6) 7.44−7.37 (4H, m), 7.37−7.34 (1H, d, *J* = 7.5 Hz), 7.28 (1H, t, *J* = 8 Hz), 7.20 (1H, d, *J* = 8 Hz), 7.19 (1H, d, *J* = 4 Hz), 7.14 (1H, br d, *J* = 8 Hz), 6.51 (1H, d, *J* = 8 Hz), 5.52 (2H, s), 3.96 (2H, s), 3.76 (3H, s).

***N*-[(3-{[3-{[(5-Chloro-2-thienyl)sulfonyl]amino}-4-(methyloxy)-1*H*-indazol-1-yl]methyl}phenyl)methyl]acetamide (7a).** A suspension of **12a** hydrochloride (2.0 g, 4.0 mmol) in DCM (10 mL) was treated with triethylamine (1.67 mL, 12.0 mmol), and the mixture was stirred for 20 min at room temperature before acetic anhydride (0.397 mL, 4.20 mmol) was added. The reaction mixture was stirred at room temperature for 2 h and then partitioned between DCM (50 mL) and saturated sodium bicarbonate solution (50 mL). The aqueous phase was extracted with DCM (30 mL), and the combined organic solutions were dried using a hydrophobic frit. The solution was evaporated under reduced pressure, and the residue was dissolved in DMSO−methanol (10 mL, 1:1) and applied to a 330 g C18 cartridge. This was eluted with a gradient of 5−40% MeCN containing 0.1% 880 aqueous ammonia and 10 mM ammonium bicarbonate over 8 CV. The required fractions were combined, and the MeCN was evaporated under reduced pressure. The residue was collected by filtration and washed with water. The solid was dried at 40 °C in vacuo for 3 d to give **7a** (1.80 g, 89%) as a white solid: LCMS (system A) RT = 0.99 min, 96%, ES +ve *m/z* 505/507 $(M + H)^+$. ^{1}H NMR δ (600 MHz, DMSO-d_6) 10.41 (1H, s), 8.28 (1H, br t, *J* = 5.5 Hz), 7.38 (1H, d, *J* = 4 Hz), 7.27 (1H, t, *J* = 8 Hz), 7.24 (1H, t, *J* = 8 Hz), 7.18 (1H, d, *J* = 8 Hz), 7.17 (1H, d, *J* = 4 Hz), 7.16 (1H, br s), 7.14 (1H, d, *J* = 8 Hz), 6.99 (1H, d, *J* = 8 Hz), 6.51 (1H, d, *J* = 8 Hz), 5.49 (2H, s), 4.19 (2H, d, *J* = 5.5 Hz), 3.76 (3H, s), 1.84 (3H, s). ^{13}C NMR δ (151 MHz, DMSO-d_6) 169.0, 153.3, 142.1, 140.1, 139.9,137.1, 135.7, 134.6, 131.9, 128.4, 127.4, 126.5, 126.4, 125.8, 109.5, 102.4, 100.1, 55.2, 51.8, 42.0, 22.5. HRMS ES +ve *m/z* 505.0770. $C_{22}H_{22}ClN_4O_4S_2$ requires 505.0771.

2-{[(3-{[3-{[(5-Chloro-2-thienyl)sulfonyl]amino}-4-(methyloxy)-1*H*-indazol-1-yl]methyl}phenyl)methyl]amino}-1,1-dimethyl-2-oxoethyl acetate (25). A suspension of **12a** (40.5 g, 87 mmol) in DCM (1 L) and triethylamine (36.4 mL, 262 mmol) was cooled down and then treated slowly with 2-chloro-1,1-dimethyl-2-oxoethyl acetate (12.52 mL, 87 mmol). The reaction mixture was stirred at room temperature under nitrogen for 80 min. It was then washed with 2 M HCl (300 mL), $NaHCO_3$ (300 mL), and brine, dried ($MgSO_4$), and evaporated under reduced pressure to give a white solid. The residue was purified by chromatography on a 1.5 kg silica column, eluting with 40−100% ethyl acetate−cyclohexane over 8 CV. The required fractions were combined and evaporated under reduced pressure to give **25** (37.87 g, 73%) as a white foam: MS ES +ve *m/z* 591/593 $(M + H)^+$. ^{1}H NMR δ ($CDCl_3$) 7.65 (1H, br s), 7.43 (1H, d, *J* = 4 Hz), 7.29−7.18 (3H, m), 7.13 (1H, br s), 7.06 (1H, br d, *J* = 7.5 Hz), 6.85 (1H, d, *J* = 8 Hz), 6.77 (1H, d, *J* = 4 Hz), 6.37 (1H, d, *J* = 8 Hz), 6.33 (1H, br t, *J* = 6 Hz), 5.43 (2H, s), 4.43 (2H, d, *J* = 6 Hz), 3.92 (3H, s), 2.05 (3H, s), 1.62 (6H, s). Additional quantities of **25** (2.16 g, 4%) were obtained from mixed fractions (3.39 g), which were further purified by chromatography on a 120 g silica column using a gradient of 40−75% EtOAc−cyclohexane over 8 CV.

***N*-[(3-{[3-{[(5-Chloro-2-thienyl)sulfonyl]amino}-4-(methyloxy)-1*H*-indazol-1-yl]methyl}phenyl)methyl]-2-hydroxy-2-methylpropanamide (6).** A solution of **25** (1.09 g, 1.84 mmol) in methanol (50 mL) was treated with potassium carbonate (0.765 g, 5.53 mmol), and the reaction mixture was stirred at room temperature. After stirring for 30 min, the reaction mixture started to crystallize, and it was allowed to stand for 2 h. It was then partitioned between water (100 mL) and ethyl acetate (150 mL). The two phases were separated, and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and evaporated in vacuo. HPLC showed presence of product in the aqueous phase, hence it was acidified with 2 M HCl and extracted with ethyl acetate (100 mL). The organic layer was washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. The combined residues were purified by chromatography on a 100 g silica column, eluting with 0−100% EtOAc−cyclohexane over 40 min. The required fractions were combined and evaporated under reduced pressure to give **6** (0.72 g, 71%) as a white solid: LCMS (system A) RT = 1.02 min, 100%, ES +ve *m/z* 549/551 $(M + H)^+$. ^{1}H NMR δ (600 MHz, DMSO-d_6) 10.40 (1H, s), 8.13 (1H, br t, *J* = 6 Hz), 7.37 (1H, d, *J* = 4 Hz), 7.26 (1H, t, *J* = 8 Hz), 7.23 (1H, t, *J* = 8 Hz), 7.17 (1H, d, *J* = 4 Hz), 7.17 (1H, d, *J* = 8 Hz), 7.14 (1H, br s), 7.13 (1H, d, *J* = 8 Hz), 6.98 (1H, d, *J* = 8 Hz), 6.50 (1H, d, *J* = 8 Hz), 5.48 (2H, s), 5.34 (1H, s), 4.22 (2H, d, *J* = 6 Hz), 3.76 (3H, s), 1.24 (6H, s). ^{13}C NMR δ (151 MHz, DMSO-d_6) 176.5, 153.3, 142.1, 140.2, 140.1, 137.0, 135.7, 134.6, 132.0, 128.4, 127.4, 126.1, 125.7, 109.5, 102.4, 100.1, 71.9, 55.2, 51.9, 41.7, 27.8. HRMS ES +ve *m/z* 549.1036. $C_{24}H_{26}ClN_4O_5S_2$ requires 549.1033.

CCR4 [^{35}S]-GTPγS Binding Assay. Membranes derived from a CHO cell line stably transfected with human CCR4 receptor (3 μg membranes per well) were adhered to Wheat Germ Agglutinin Polystyrene LEADSeeker scintillation proximity assay beads (250 μg/well) in assay buffer containing *N*-2-hydroxyethylpiperazine-*N*′-2-

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ethanesulfonic acid (20 mM), $MgCl_2$ (10 mM), NaCl (100 mM), saponin (40 μg/mL), bovine serum albumen (0.05% w/v), GDP (4.4 μM), and pH adjusted to 7.4 using 1 M KOH. After 1 h precoupling at 4 °C, the bead/membrane suspension was mixed in a 4:5 ratio with [^{35}S]-GTPγS (PerkinElmer LAS UK Ltd., radioactivity concentration 37 MBqmL^{-1}; specific activity = 1250 Cimmol^{-1}) made up in assay buffer containing MDC (at a concentration that results in the final assay concentration of MDC being EC_{80}) to give a final radioligand concentration of 0.6 nM. A 45 μL/well aliquot of this reagent mixture was dispensed into white Greiner polypropylene 384-well plates containing stock solution of the test compounds in DMSO (10 mM, 0.5 μL) or 0.5 μL of DMSO as a control, and the assay plates were sealed and centrifuged at 1000 rpm for 20 s. The plates were left to equilibrate in darkness for between 3 and 6 h before reading on a ViewLux luminescence imager using a 613/55 filter for 5 min/plate. Data were analyzed using a four-parameter logistical equation to determine the antagonist IC_{50}.

Human Whole Blood F-Actin Polymerization Assay. Volunteers gave informed consent for blood donation and denied taking any medication in the 7 days prior to donation. Blood (9 volumes) was taken from healthy human subjects into 3.8% sodium citrate solution (1 volume). The blood was incubated at room temperature with saturating concentrations of fluorescein isothiocyanate (FITC)-conjugated mouse antihuman CD4 and a noninactivating phycoerythrin (PE)-conjugated mouse antihuman CCR4 (BD Biosciences) or appropriate isotype control antibodies for 10 min. The blood was then incubated with antagonists or vehicle (0.1% DMSO) at 37 °C for 30 min before addition of the agonist (MDC from ALMAC Sciences, UK; TARC from PeproTech EC, UK) for 15 s. The assay was terminated by adding 10 volumes of FACS Lysing solution (BD Biosciences). After 30 min, the cells were centrifuged (1000*g* for 5 min), resuspended in FACS Lysing solution (200 μL), and incubated at room temperature for a further 15 min. The cell suspensions were then centrifuged (1200*g* for 5 min), washed twice by resuspending in PBS (150 μL), and centrifuging as above to recover the cells and incubated for 20 min with lysophosphatidylcholine (100 μg.mL^{-1}) and Alexa fluor 647 phalloidin (0.075 units.mL^{-1}). The cells were centrifuged at 1200*g* for 5 min and resuspended in PBS. The F-actin content of the $CD4^+$ $CCR4^+$ lymphocytes in each sample was determined on a FACSCalibur flow cytometer by measuring the mean Alexa fluor 647 (FL-4) fluorescence intensity of 500 cells. This was expressed as a fraction of the Alexa fluor 647 fluorescence intensity of the $CCR4^-$ lymphocytes in the same sample.

Single Crystal X-ray Diffraction Studies of 57 and 58. Crystal structures of **57** and **58** were determined at 150K using Mo Kα X-radiation (λ = 0.71073 Å).

Summary of the structure determination of **57**: $C_{13}H_{12}ClN_3O_3S_2$, M = 357.83, monoclinic, space group $P2_1/n$ (alt. $P2_1/c$, no. 14), a = 11.034(2) Å, b = 8.6297(17) Å, c = 16.101(3) Å, β = 94.54(3)°, V = 1528.3(5) Å^3, Z = 4, D_{calc} = 1.555 Mgm^{-3}, $F(000)$ = 736, μ(Mo Kα) = 0.538 mm^{-1}; $R1$ [$I > 2\sigma(I)$] = 0.0358; $wR2$ (all data) = 0.0733; and S = 1.061.

Summary of the structure determination of **58**: $C_{16}H_{12}ClN_3O_2S_3$, M = 409.92, monoclinic, space group $P2_1/c$ (no. 14), a = 10.360(2) Å, b = 17.010(3) Å, c = 10.076(2) Å, β = 98.05(3)°, V = 1758.2(6) Å^3, Z = 4, D_{calc} = 1.549 Mgm^{-3}, $F(000)$ = 840, μ(Mo Kα) = 0.589 mm^{-1}; $R1$ [$I > 2\sigma(I)$] = 0.0608; $wR2$ (all data) = 0.1071; and S = 1.056.

Full details of the structure determinations can be found in the Supporting Information. In addition, crystallographic data (excluding structural factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 906759 and 906760. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@CCDC.cam.ac.uk)."

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparative details and spectroscopic data for compounds: **10b**, **11b**, **12b**, **7b**, **9c**, **10c**, **11c**, **7c**, **9d**, **10d**, **11d**, **12d**, **7d**, **12e**, **7e**, **9f**, **13**−**18**, **7f**, **19**−**21**, **7g**, **7h**, **7i**, **7j**, **22**, **23**, **7k**, **24a**−**24l**, **31**, **32**, **26**−**28**, **35**−**39**, **33a**, **33b**, **42**−**47**, **40a**, **40b**, **50**, **52**, **53**, **48**, **54**, **55**, **56a**, **56b**, **56c**, **56d**, **57**, **58**, and solubility determinations. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CCR4, CC-chemokine receptor 4; TARC, thymus activation-regulated chemokine; MDC, macrophage-derived chemokine; [^{35}S]-GTPγS, guanosine-5′-[γ-thio]triphosphate; MDCK, Madin Darby canine kidney; Cl, clearance; Vd, volume of distribution; PK, pharmacokinetics

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