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Lead optimisation of the *N*1 substituent of a novel series of indazole arylsulfonamides as CCR4 antagonists and identification of a candidate for clinical investigation

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ABSTRACT

Synthesis and preliminary SAR of the *N*1 substituent of a novel series of indazole sulfonamide chemokine receptor 4 (CCR4) antagonist is reported. Compound **7r** was identified for further development.

Chemokines are a group of small chemotactic proteins of 8–10 kDa, which possess four conserved cysteines.[1] More than 50 chemokines have been identified, and 17 chemokine receptors have been described in humans. Chemokines are classified into four groups designated CC, CXC, C and CX_3C based on the arrangement of the first two conserved cysteines located at or near the N-terminus of each protein.[2] Chemokines exert their effects by binding to cell surface receptors, which belong to the seven transmembrane G-protein-coupled receptor family, and play an important role in immune responses by directing leucocytes to inflamed tissues. Ten CC chemokine receptors have been identified so far and named as CC-chemokine receptor 1, 2 etc. Most chemokine receptors recognise more than one chemokine and several chemokines bind to more than one receptor.[3] CCL17, also known as thymus activation-regulated chemokine (TARC) and CCL22, also known as macrophage-derived chemokine (MDC), are a pair of CC chemokines that bind to CC-chemokine receptor 4 (CCR4).[2] CCR4 is mainly expressed in T helper 2 (Th2) 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 are believed to contribute to the pathological consequences of allergic diseases.[5] 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] It has therefore been suggested that interaction between CCR4 and its ligands plays an important role in asthma. CCR4 antagonists represent a novel therapeutic intervention in diseases where CCR4 has a central role in pathogenesis. Progress in the discovery of small-molecule CCR4 antagonists as immunomodulatory agents was reviewed by Purandare and Somerville in 2006.[8] A number of publications on CCR4 antagonists have appeared in the literature since the last review[9–18] and these antagonists are classed into two broad chemo types (Fig. 1). The first chemo type includes lipophilic amines such as Bristol Myers Squibb compound **1**,[10] Pfizer compound **2**,[11] Astellas compound **3**,[13] and the Daiichi Sankyo compound **4**.[17] The second chemo type is a series of pyrazine arylsulfonamides, such as the AstraZeneca compound **5**[19] and Ono compound **6**.[20] In this communication we are disclosing our own studies on the identification and the lead optimisation of the *N*1 substituent of a novel class of indazole arylsulfonamides **7**.

The assays used in this study were a [^{35}S]-GTPγS competition assay and/or a ^{125}I-TARC radioligand binding assay both using

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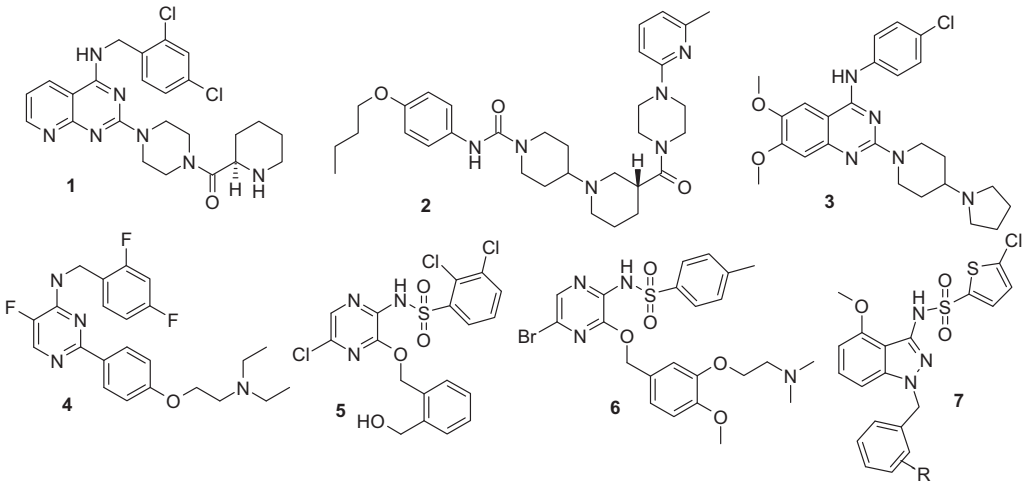
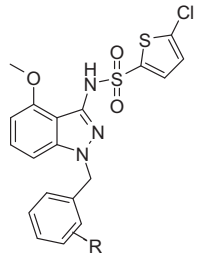


Figure 1. Structures for some recently published CCR4 antagonists.

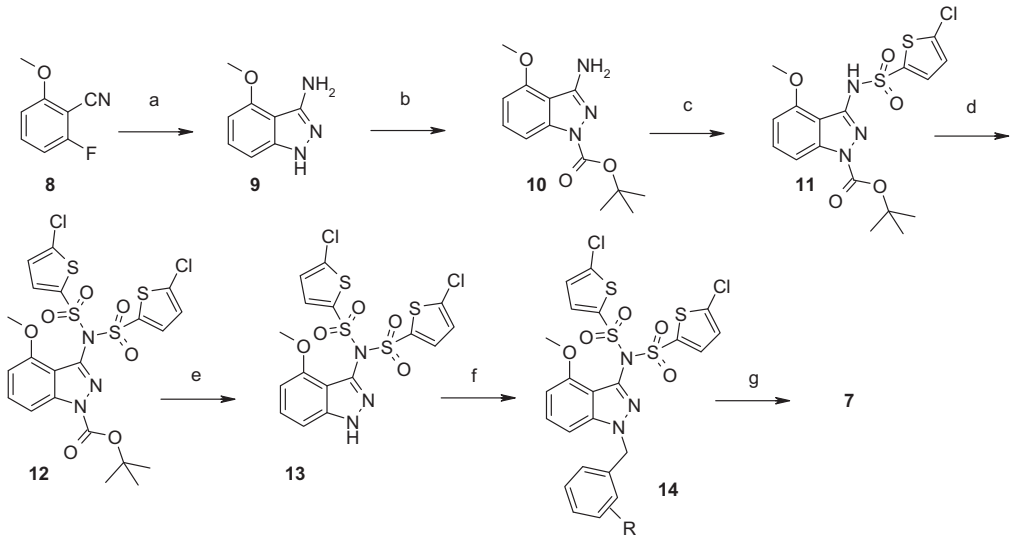
recombinant human CCR4-expressing CHO cell membranes bound to SPA beads with output measured on a Wallac Microbeta Trilux scintillation counter. Our lead molecule **7** (R = H) was identified from a focused pharmacophore array approach and had a pIC_{50} of 6.4 in the GTPγS assay and 6.6 in the TARC assay. By comparison the BMS compound **1** and Ono compound **6**, which were used as standards, had pIC_{50} of 8.3 and 8.4, respectively, in the GTPγS assay, and 9.1 and 8.5, respectively, in the TARC assay (Table 1). Lead optimisation started by investigating substitution with Cl-, F-, Me-, CN- and MeO-groups, and attempting to identify the position on the benzyl group of **7a** which tolerated a substituent. These analogues were synthesised by the routes shown in Schemes 1 and 2, depending on the commercial availability of the benzyl substituent.[21] The synthetic route outlined in Scheme 1 started with reaction of 2-fluoro-6-methoxybenzonitrile (**8**) with hydrazine hydrate in 1-butanol at reflux. The resulting 3-amino-indazole **9** was protected at the *N*1 position by reacting with di-*tert*-butyl dicarbonate in the presence of triethylamine and DMAP to give **10**. Reaction of **10** with one equivalent of 5-chloro-2-thiophenesulfonyl chloride gave a mixture of mono-sulfonylated

Table 1
In vitro data pIC_{50} for GTPγS and pK_i for I^{125} TARC SPA binding assays, CLND solubility, lipophilicity (daylight *c*log*P*), and human whole blood TARC induced F-actin polymerisation pA_2

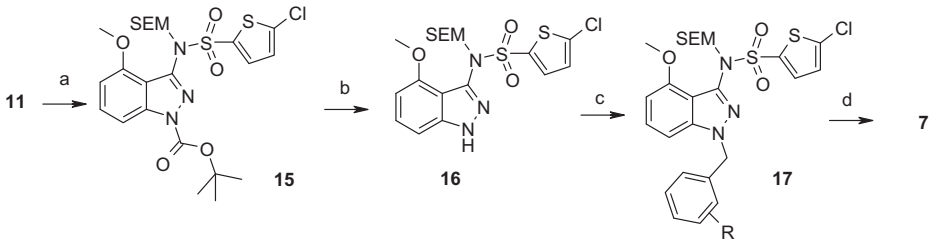


Compd	R	GTPγS pIC_{50}	I^{125} TARC SPA pK_i (*n*)	CLND solub. (μg/mL)	*c*log*P*	hWB actin polym. pA_2 (*n*)
1	NA	8.26 ± 0.01 (131)	9.1 ± 0.1 (11)	145	4.9	7.3 ± 0.3 (4)
6	NA	8.41 ± 0.02 (127)	8.5 ± 0.1 (8)	170	4.4	6.6 ± 0.1 (22)
7a	H–	6.41 ± 0.01 (2)	6.6 ± 0.1 (10)	1	4.6	5.2 ± 0.2 (4)
7b	2-MeO–	ND	6.8 ± 0.1 (2)	12	4.5	ND
7c	3-MeO–	6.8 ± 0.1 (2)	6.9 ± 0.1 (2)	12	4.5	ND
7d	4-MeO–	ND	6.6 ± 0.2 (2)	7	4.5	ND
7e	3,4-Di-MeO–	7.64 ± 0.02 (5)	7.7 ± 0.1 (5)	46	4.3	5.6 ± 0.1 (2)
7f	2-CH_2OH	6.49 ± 0.02 (2)	6.6 ± 0.0 (2)	74	3.5	ND
7g	3-CH_2OH	7.49 ± 0.13 (2)	7.4 ± 0.0 (2)	127	3.6	5.2 ± 0.2 (2)
7h	4-CH_2OH	7.32 ± 0.03 (2)	7.3 ± 0.0 (2)	123	3.6	ND
7i	3-CH_2NH_2	7.04 ± 0.09 (2)	7.0 ± 0.1 (2)	17	3.6	6.0 ± 0.1 (3)
7j	3-$CONH_2$	7.26 ± 0.03 (5)	7.4 ± 0.1 (5)	140	3.1	5.8 ± 0.2 (6)
7k	3-CONHMe	7.1 ± 0.1 (2)	6.5 ± 0.1 (2)	167	3.4	ND
7l	3-$CONMe_2$	6.38 ± 0.08 (2)	5.9 ± 0.1 (2)	160	3.1	ND
7m	3-$CH_2NHCOCH_3$	7.50 ± 0.04 (10)	7.9 ± 0.0 (2)	124	3.4	6.0 ± 0.1 (7)
7n	3-$CH_2NMeCOCH_3$	6.89 ± 0.03 (2)	ND	151	3.5	ND
7o	4-$CH_2NHCOCH_3$	ND	6.8 ± 0.1 (2)	104	3.4	ND
7p	3-$CH_2NHCOCH_2OH$	7.56 ± 0.06 (2)	7.8 ± 0.2 (2)	167	3.1	5.7 ± 0.1 (2)
7q	3-$CH_2NHCOCH(Me)OH$	7.9 ± 0.1 (3)	8.1 ± 0.1 (2)	146	3.4	6.2 ± 0.0 (2)
7r	3-$CH_2NHCOCMe_2OH$	7.83 ± 0.02 (95)	8.0 ± 0.0 (2)	162	3.7	6.2 ± 0.1 (11)

NA not applicable. ND not determined. When n <3 SEM is the SD.



Scheme 1. Reagents and conditions: (a) $NH_2NH_2{\cdot}H_2O$, ^nBuOH, 92%; (b) BOC_2O, Et_3N, DMAP, DCM, 67%; (c) 5-chloro-2-thiophenesulfonyl chloride (1 equiv), pyridine, DCM, 22%; (d) 5-chloro-2-thiophenesulfonyl chloride (3 equiv), pyridine, DCM, 45 °C, 3 days, 80%; (e) TFA, DCM, 100%; (f) substituted benzyl alcohol, PPh_3, $^iPrO_2CN{=}NCO_2Pr^i$, THF, 70 °C, 20–80%; (g) 2 M NaOH, H_2O, MeOH, 50 °C, 52–85%.



Scheme 2. Reagents and conditions: (a) SEM-Cl, DIPEA, DCM, 100%; (b) Na_2CO_3, H_2O, DMF, 85 °C, 74%; (c) $ArCH_2Br$, K_2CO_3, DMF, 44–88%; (d) $^nBu_4NF$, THF, 90–95%.

product **11**, and bis-sulfonylated product **12**, which were separable by chromatography. Treatment of **10** or **11** with excess sulfonyl chloride gave **12**, which was then de-protected with TFA to give **13**. Mitsunobu reaction of **13** with a variety of benzyl alcohols gave the *N*1 alkylated product **14**, accompanied by a small amount of *N*2 alkylation, which was readily removed by chromatography. Hydrolysis of **14** with aqueous sodium hydroxide in methanol gave target test compounds **7**. The synthetic route outlined in Scheme 2 commenced with reaction of **11** with SEM-Cl to give **15**, removal of the BOC protecting group to give **16**, alkylation of **16** with an appropriate benzyl halide to give **17**, and finally cleavage of the SEM protecting group to give **7**. It was possible to use benzyl bromides instead of benzyl alcohols to produce compounds **14** by the method outlined in Scheme 1, however, it was not possible to use benzyl alcohols to produce compounds **17** by the method outlined in Scheme 2. Analogues bearing Cl-, F-, Me-, CN-, and MeO-groups were very lipophilic (*c*log*P* 4.6–5) and had very low solubility as measured by ChemiLuminescent Nitrogen Detection (CLND) solubility assay (<10 μg/mL). This high throughput kinetic solubility assay involves addition of aqueous buffer to a test compound DMSO solution over a period of time until the compound precipitates. The potency of these lipophilic compounds (not shown in Table 1) was not significantly different from that of **7a**, apart from the MeO-substituted analogues (Table 1), which highlighted a small preference for *meta*-substitution (**7c** pIC_{50} 6.9), and in the case of the 3,4-disubstituted analogue **7e** a significant increase in potency (pIC_{50} 7.7). Since the solubility of all of the above analogues was very low (7–46 μg/mL), lead optimisation focused on the introduction of solubilising groups on the *N*1 benzylic moiety.

The three regioisomeric analogues bearing a hydroxymethyl substituent (**7f**, **7g** and **7h**) were prepared by the method shown in Scheme 1, and for all three analogues their solubility increased to >70 μg/mL. The *meta*-isomer (**7g**) and *para*-isomer (**7h**) were more potent than the *ortho*-isomer (**7f**). Alcohol **7g** had similar activity to **7a** on TARC-induced increases in the F-actin content (a cytoskeletal reorganisation event of chemotaxis) of human $CD4^+$ $CCR4^+$ lymphocytes measured in human whole blood (pA_2 5.2). Introduction of the *meta*-aminomethyl moiety (**7i**) increased the whole blood potency even further to 6.0, despite a drop in its GTPγS potency to 7.0. The solubility of **7i** was also reduced to 17 μg/mL and this might be due to the zwitterionic character of the compound. The primary benzamide **7j** was more potent than the secondary amide **7k**, which in turn was more potent than the tertiary amide **7l** in the TARC assay, which is consistent with the requirement for a hydrogen-bond donor. In the GTPγS the tertiary amide **7l** was again less potent, however, the primary and secondary amides were equipotent in this assay. The solubility of all three benzamides increased to 140–167 μg/mL, and furthermore the whole blood activity of **7j** was 5.8, similar to that of **7i**. The acetamide **7m** was prepared by acetylation of **7i** with acetic anhydride, and was one of the more potent analogues in vitro (7.9 in the TARC assay, and 7.5 in the GTPγS assay). In addition **7m** was potent in the whole blood assay (pA_2 6.0) making it a candidate for further investigation in pharmacokinetic studies. The *N*-methyl acetamide **7n** was significantly less potent, further supporting the hypothesis that a hydrogen-bond donor was preferred for an interaction with the receptor. The *para*-acetamide analogue **7o** was about ten-fold less potent than **7m** (6.8 vs 7.9 in the TARC assay confirming the

Table 2
CD rat and Beagle dog pharmacokinetic data for **7m** and **7r**

Species		7m	7r
CD Rat	iv Dose (mg/kg)	1.0	1.0
	po Dose (mg/kg)	1.0	1.0
	n Value (iv/po)	2/2	2/2
	Cl (mL/min/kg)	5	15
	Vdss (L/kg)	0.57	1.9
	$T_{½}$ (h)	1.6	2.5
	F%	Complete	85
Beagle dog	iv Dose (mg/kg)	0.54	0.55
	po Dose (mg/kg)	1.1	1.1
	n Value (iv/po)	4/4	4/4
	Cl (mL/min/kg)	18 ± 5	9.0 ± 1.9
	Vdss (L/kg)	3.2 ± 1.1	2.2 ± 0.8
	$T_{½}$ (h)	1.6 ± 0.2	2.6 ± 0.5
	F%	49 ± 5	97 ± 27

meta-position as the preferred site for substitution of the benzylic ring. The solubility of **7m** was 124 μg/mL, therefore, increasing the solubility of subsequent analogues by introducing solubilising groups became our next target. The analogues **7p**, **7q** and **7r** bearing a hydroxyl group were synthesised by amide coupling of **7i** with the appropriate carboxylic acid.[21] Analogues **7q** and **7r** provided increased potency, and whole blood activity. The tertiary alcohol **7r** had slightly lower potency than the two reference compounds **1** and **6** in the GTPγS assay, lower lipophilicity (*c*log*P* 3.7), and whole blood activity of 6.2. The permeability of compounds **7m** and **7r** in MDCK cells was measured as 254 and 132 nm/s, respectively, and was sufficiently high to progress the compounds to pharmacokinetic studies. 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. Compounds **7m** and **7r** were studied in vivo in rat and dog, and the data are shown in Table 2. In the rat the acetamide **7m** had complete oral bioavailability, low volume of distribution and low clearance [6% liver blood flow (LBF)]. In the dog bioavailability was reduced to 49%, volume was moderate, however, the clearance increased substantially to 58% LBF. In the rat the α-hydroxy-isobutyramide **7r** had high oral bioavailability, moderate volume and low clearance (18% LBF), and a half-life of 2.5 h which was longer than **7m**. The bioavailability of **7r** in the dog was 97% and was higher than **7m**. The volume of **7r** was moderate, however, its clearance (29% LBF) was lower than **7m**, resulting in a longer half-life (2.6 h). In contrast the reference compound **1** had negligible oral bioavailability in an in-house rat PK study, and was also reported to have been dosed subcutaneously, suggesting low oral bioavailability.[10] Based on its pharmacokinetic profile **7r** was selected for further progression and the first time in human clinical data will be reported shortly.[22]

No in vivo pharmacodynamic data were generated for **7r** due to the differences in immunology between animals and human, the complexity of chemokine systems across species, and the lack of convincing evidence that CCR4 can drive inflammation in animal models of asthma. Greater relevance was placed on in vitro studies using target human CD4+ CCR4+ and CD45RO+ T cells and biopsies taken from atopic asthmatics, supernatants from which were used to induce chemotaxis of autologous memory T-cells. A more comprehensive SAR report together with additional studies on **7r** will be published in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.104. These data include MOL files and InChiKeys of the most important compounds described in this article.

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