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Identification of novel azaindazole CCR1 antagonist clinical candidates

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ABSTRACT

Exploring various cyclization strategies, using a submicromolar pyrazole HTS screening hit **6** as a starting point, a novel indazole based CCR1 antagonist core was discovered. This report presents the design and SAR of CCR1 indazole and azaindazole antagonists leading to the identification of three development compounds, including **19e** that was advanced to early clinical trials.

Introduction

Chemotactic cytokine receptor 1 (CCR1) is a G protein-coupled receptor that belongs to a family of > 20 chemokine receptors that have emerged as attractive targets for drug discovery.[1,2] Various chemokines interact with these chemokine receptors that are well known to mediate basal and inflammatory leukocyte trafficking. CCR1 is expressed on immune cells including monocytes, macrophages, *T*-lymphocytes, neutrophils, basophils, eosinophils, NK cells, mast cells and dendritic cells. The binding of the chemokines MIP-1α (CCL3), MCP3 (CCL7) and RANTES (CCL5) to CCR1 is reported to play a role in the trafficking of monocytes, macrophages and Th_1 cells to the site of inflammation in rheumatoid arthritis (RA) and multiple sclerosis (MS). Additionally, the chemokines are all found in the Central Nervous System (CNS) of MS patients, and MIP-1α and RANTES are found in the CNS of rodents in the experimental autoimmune encephalomyelitis (EAE) disease relevant model. Macrophages and Th_1 cells in the synovia of RA patients are also major producers of MIP-1α and RANTES, and they recruit leukocytes to the synovial tissues of RA patients resulting in chronic inflammation. Thus, CCR1 has been regarded as a potential target for the treatment of inflammatory disease. Antagonists that block the interactions between CCR1 and its chemokine ligands could block chemotaxis of monocytes, macrophages and Th_1 cells to the site of inflammation, ameliorating the chronic inflammation associated with autoimmune diseases such as RA and MS.[3,4] Despite set-backs in the use of CCR1 antagonists in autoimmune disease and inflammation, pharmacological intervention to disease via CCR1 remains of strong interest, for example for the treatment of systemic fungal infection or in oncology.[5,6]

A number of publications have disclosed ligands as antagonists of CCR1 and several have shown promising results in animal models of disease.[7–11] However, several early clinical candidates have failed to show efficacy (Fig. 1):[12] BX-471 (**1**) failed to show efficacy in a Phase II clinical trial in patients with relapsing remitting MS.[13–15] CP-481715 (**2**) failed to show efficacy in RA patients after 6 weeks of treatment. Compound **1** and **2** may not have achieved sufficient inhibition of the receptor since their PK profile led to high human doses. Millennium compound MLN-3897 (**3**) was also stopped in the clinic after lack of efficacy in a Phase II clinical trial for RA.[16–18] It is presumed that compound **3** could not be dosed sufficiently high due to safety

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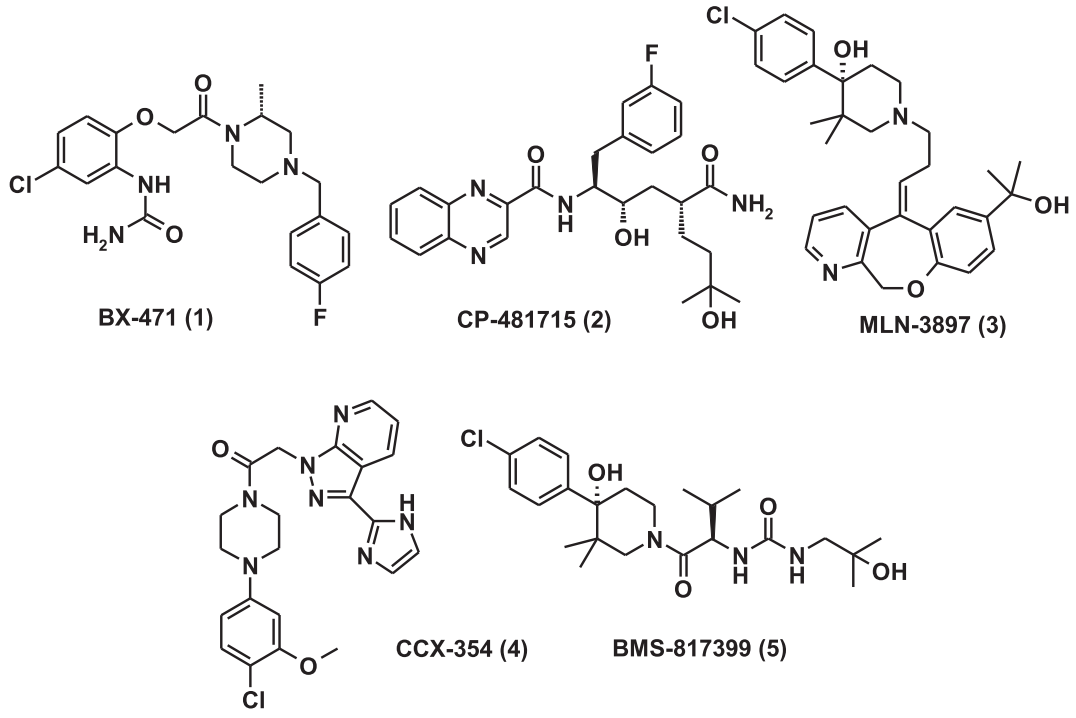


Fig. 1. Clinical CCR1 antagonists.

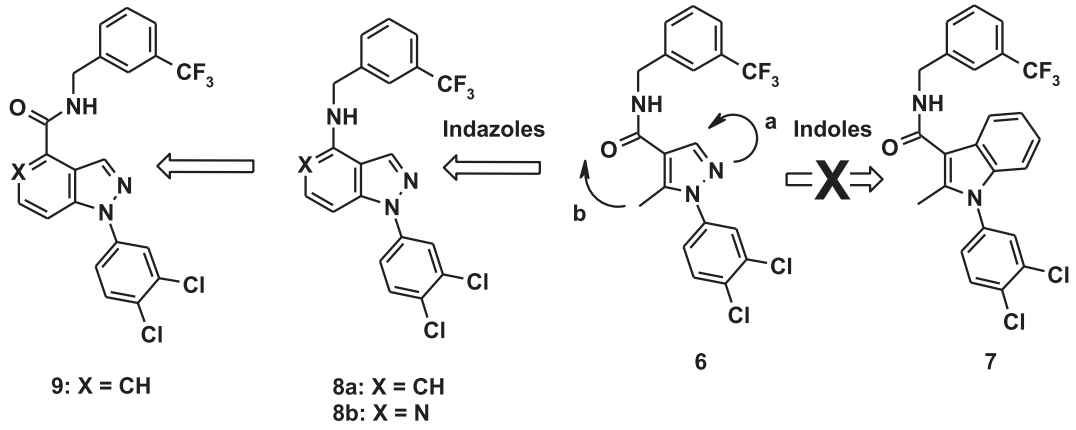
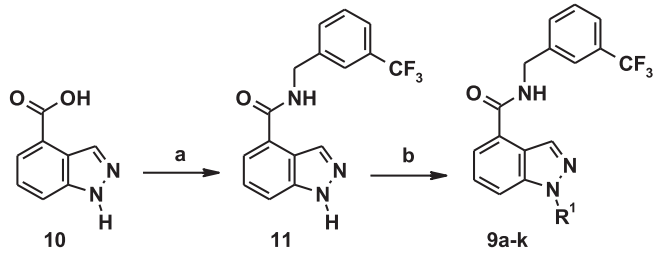
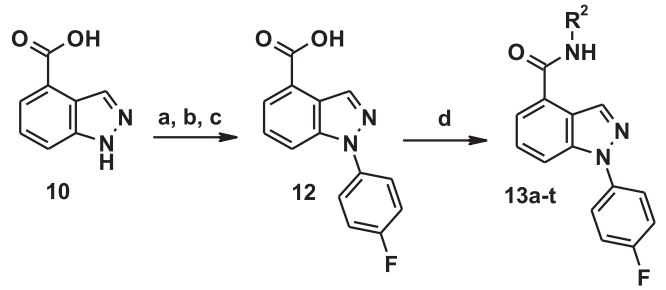


Fig. 2. Cyclization strategy leading to indazole scaffold.



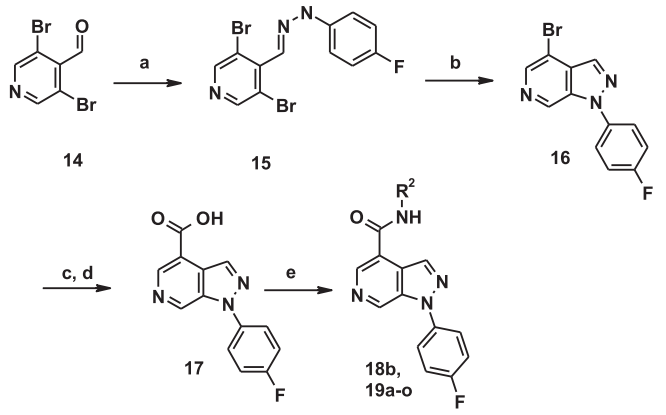
Scheme 1. Reagents and conditions: (a) PyBop, Et_3N, 3-trifluoromethylbenzyl amine (b) R^1-I, CuI, K_2HPO_4, *trans-N,N'*-dimethylcyclohexane-1,2-diamine, toluene or DMF.



Scheme 2. Reagents and conditions: (a) $TMSCH_2N_2$, MeOH, toluene or; methanolic HCl, Δ (b) 4-fluoroiodobenzene, CuI, K_2HPO_4, *trans-N,N'*-dimethylcyclohexane-1,2-diamine, toluene or DMF (c) KOH, $MeOH/H_2O$ (d) PyBop, Et_3N, R^2NH_2.

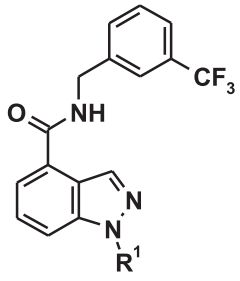
limitations. In 2012, ChemoCentryx reported positive proof-of-clinical-concept for an oral CCR1 antagonist CCX354 (**4**; dose 200 mg, once daily) in a 12-week Phase II trial in patients (n = 160) with RA (ACR20 response at 12 weeks = 56%).[19–22] In contrast, in 2014, results from a 12-week Phase II trial in patients (n = 123) with RA with BMS-817399 (**5**) did not show statistically significant differences in disease scores (ACR20, DAS28) or MRI synovitis compared to placebo despite achieving excellent coverage of the receptor.[23] Additional compound classes have been described and have advanced into clinical trials.[24,25]

In the immediately preceding publication in this issue, a novel



Scheme 3. Reagents and conditions: (a) 4-fluorophenylhydrazine·HCl, NaOAc, ethanol/water (b) CuI, *trans-N,N'*-dimethylcyclohexane-1,2-diamine, K_2CO_3, NMP (c) CO, $Pd(PhCN)_2Cl_2$, dppf, Et_3N, EtOH (d) KOH, MeOH/H_2O (e) PyBop, Et_3N, R^2NH_2.

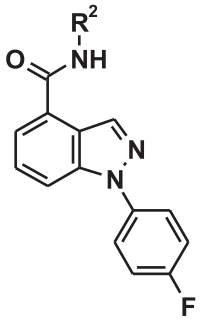
Table 1
N-1 substituted indazoles.

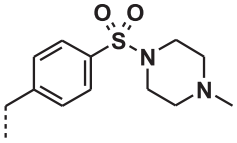
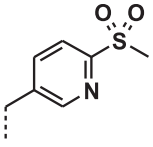


Compound	R^1	CCR1 Ca^{2+} flux IC_{50} (nM)
9a	3,4-dichlorophenyl	260
9b	benzyl	> 3000
9c	phenyl	750
9d	4-chlorophenyl	210
9e	4-fluorophenyl	22
9f	4-cyanophenyl	> 3000
9g	4-methoxyphenyl	> 3000
9h	2-fluorophenyl	2500
9i	3-fluorophenyl	1100
9j	methyl	> 3000
9k	4-fluoro-3-pyridyl	17

pyrazole scaffold **6**, identified via a HTS screen, was disclosed as an antagonist of CCR1 (Fig. 2).[26,27] During the lead identification phase of the program, various approaches were considered to identify additional chemical matter, prior to transitioning to lead optimization. For various reasons, which included achieving balance of the overall drug-like properties and potency, the pyrazole scaffold compounds were de-prioritized as development candidates. A number of cyclization strategies were considered, using the pyrazole scaffold as an attractive template for scaffold hopping. Several of these strategies yielded a 5,6-fused aromatic ring system, providing an additional ring in the core to afford new opportunities for SAR and the optimization of drug-like properties. A cyclization via mode "a" would provide indole compounds like **7**. The Intellectual Property assessment was unfavorable for these compounds. However, cyclization mode "b" via the methyl group and the amide carbonyl oxygen, where the carbonyl double bond is incorporated in the ring and the carbonyl oxygen is mimicked by X (where X = CH or N), afforded indazole compounds like **8**. Compound **8a** (X = CH) could generate a potentially genotoxic aromatic amine moiety through hydrolysis as a potential degradation product or

Table 2
Benzylamide SAR.



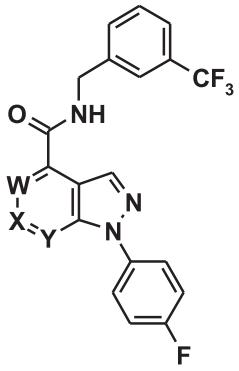
Compound	R^2	CCR1 Ca^{2+} flux IC_{50} (nM)	High-Throughput Solubility pH 7 (μg/mL)
9e	3-(trifluoromethyl)-benzyl	22	< 0.1
13a	2-(trifluoromethyl)-benzyl	1200	< 0.1
13b	3-chlorobenzyl	450	< 0.1
13c	3-methoxybenzyl	> 3000	0.2
13d	3-(methoxycarbonyl)-benzyl	1700	< 0.1
13e	3-carboxybenzyl	> 3000	> 100
13f	3-(*N*-methylaminocarbonyl-benzyl	> 3000	NT
13g	3-(methylsulfonyl)-benzyl	61	32
13h	3-(*N*-methylaminosulfonyl)-benzyl	55	NT
13i	3-(*N*-ethylaminosulfonyl)-benzyl	170	0.9
13j	3-(*N*-isopropylaminosulfonyl)-benzyl	180	0.7
13k	3-(*N,N*-dimethylaminosulfonyl)-benzyl	44	< 0.1
13l	4-(methylsulfonyl)-benzyl	68	< 0.1
13m	4-(*N*-methylaminosulfonyl)-benzyl	4.5	2.6
13*n*	4-(*N*-isopropylaminosulfonyl)-benzyl	5.0	0.7
13o	4-(*N,N*-dimethylaminosulfonyl)-benzyl	6.0	0.2
13p		12	5.5
13q	(2-pyridyl)-methyl	> 3000	NT
13r	(3-pyridyl)-methyl	> 3000	NT
13s	(4-pyridyl)-methyl	1200	NT
13t		120	21

metabolite. This structural alert could be lessened for structure **8b** (X = N), and the SAR from the pyrazole series indicated that the amide motif was critical for potency. Thus, compound **9** (X = CH) was prepared as the initial analogue and found to have modest CCR1 antagonist activity (IC_{50} = 260 nM in a MIP-1α-induced calcium flux assay in CCR1-expressing cells).[28]

Scheme 1 shows how indazoles **9** with various substituents on the indazole nitrogen were prepared.[29] Commercially available indazole-4-carboxylic acid (**10**), was coupled with 3-trifluoromethylbenzyl amine to afford amide **11**. A copper mediated coupling of various aryl or heteroaryl iodides afforded analogs **9a–k**.

To prepare compounds with variation of the benzylamine moiety, the carboxylic acid **10** was esterified (Scheme 2). A copper mediated coupling of 4-fluoroiodobenzene followed by ester hydrolysis gave the

Table 3
Azaindazole core SAR.



Compound	W, X, Y	CCR1 Ca^{2+} flux IC_{50} (nM)	High-Throughput Solubility pH 7 (μg/mL)
9e	W, X, Y = CH	22	< 0.1
18a	W = N; X, Y = CH	450	2.1
18b	W = CH; X = N; Y = CH	3.0	0.1
18c	W = N; X = CH; Y = N	> 3000	NT

carboxylic acid **12**. Activation of the carboxylic acid in the presence of the benzyl amine provided amide analogues **13a–t**. Certain substituents (such as the alkylsulfones and sulfonamides) had to be introduced or elaborated post coupling.[29] Chiral amines were prepared using Ellman's sulfinamide chemistry from the corresponding bromopyridine.[29–31]

The 6-azaindazoles **18b**, **19a–o** were synthesized through copper catalyzed cyclization of an arylhydrazone **15** derived from 3,5-dibromopyridyl-4-carboxaldehyde (**14**) to give the 4-bromo-6-azaindazole **16** (Scheme 3). Compound **16** was subjected to Pd-catalyzed carbonylation and hydrolysis of the ester to give the carboxylic acid **17**. The acid **17** was coupled with the appropriate benzylamines as in Scheme 2 to generate 6-azaindazole analogues **18b, 19a–o**.

The 5-azaindazole and 5,7-diazaindazole synthesis has been described previously.[29] Certain substituents (such as the alkylsulfones and sulfonamides) had to be introduced or elaborated post coupling.[32] Chiral amines were prepared using Ellman's sulfinamide chemistry from the corresponding bromopyridine.[29–31]

As shown in Table 1, a number of *N*-1 substituted indazoles were surveyed. Benzyl substitution was not tolerated as in **9b**. The unsubstituted phenyl compound **9c** was less potent than the original lead **9a**. The 4-chlorophenyl analog **9d** was equipotent to **9a**, however the 4-fluorophenyl analog **9e** (CCR1 IC_{50} 22 nM) was 10-fold more potent than **9a**. Substitution by larger groups at the 4-position was not tolerated, as seen in examples **9f** and **9g**, each having a CCR1 IC_{50} > 3000 nM. *Ortho*- and *meta*-fluoro substituted compounds **9h** and **9i** displayed low micromolar activity in the calcium flux assay and were 50 to 100-fold less potent than the *para*-isomer **9e**. A number of small alkyl R^1 groups were prepared (e.g. methyl as in **9j**) which were not active at the highest concentrations tested in the CCR1 calcium flux assay. Interestingly, the 4-fluoro-3-the pyrazole series where no polar atoms were tolerated in this area of the binding pocket (see immediately preceding publication in this issue).

Next, the benzylamine moiety SAR was explored. As shown in Table 2, several key substitution patterns emerged. In general, monosubstitution in the 2-position had a detrimental effect on potency in the CCR1 assay, exemplified by the *ortho*-CF_3 substituted compound **13a**. Substitution in the 3-position was better tolerated, however compounds containing groups such as 3-chloro as in **13b** and 3-methoxy as in **13c** lost significant potency (CCR1 IC_{50} 450 nM and > 3000 nM, respectively). The ester **13d** retained some micromolar activity, while the carboxylic acid **13e** and the *N*-methyl carboxamide **13f** had CCR1 IC_{50} values > 3000 nM. The 3-methylsulfone **13g** displayed acceptable potency in the CCR1 calcium flux assay (IC_{50} 61 nM). Furthermore, compound **13g** had appreciable solubility in a high-throughput kinetic solubility assay (32 μg/mL) compared to compound **9e** (< 0.1 ug/mL). The sulfonamides **13h–k** also generally demonstrated acceptable potency. The *N*-methylsulfonamide **13h** (CCR1 IC_{50} 55 nM) and the *N*,*N*-dimethylsulfonamide **13k** (CCR1 IC_{50} 44 nM), were of similar potency suggesting the NH was not acting as a hydrogen bond donor. The *N*-ethylsulfonamide **13i** and the *N*-isopropylsulfonamide **13j** were 3-fold less potent than **13h** suggesting steric bulk may be undesirable for potency in the calcium flux assay. As observed in the 3-position, the 4-methylsulfone **13l** (CCR1 IC_{50} 68 nM) was more potent than compounds with other groups in the 4-position. While the 3- and 4-methylsulfones were equipotent, in contrast, the 4-sulfonamides **13m–o** were 10- to 40-fold more potent than the corresponding 3-sulfonamides **13h**, **13j**, and **13k**, respectively. In general, the sulfonamide analogs in Table 2 displayed lower aqueous solubility than the methyl sulfone analogs. Interestingly, potent activity could be retained in the sterically demanding, but also more basic *N*-methylpiperidinesulfonamide **13p**.

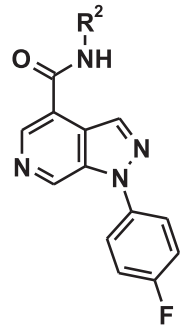
It should also be noted that the amide NH is critical for CCR1 activity. *N*-alkylation of the amide nitrogen of **13l** with methyl, ethyl or propyl groups gave significantly reduced potency in the calcium flux assay.

To further improve aqueous solubility, the SAR of the amide moiety was expanded to include various pyridyl substitution patterns. The unsubstituted pyridyl groups in **13q–s** were not tolerated; solely the 4-pyridyl analogue **13s** retained detectable CCR1 activity (CCR1 IC_{50} 1200 nM). However, in combination with methylsulfone substitution as in **13t** the compound maintained potent activity (CCR1 IC_{50} 120 nM). This finding was explored further and will be discussed in the context of the azaindazoles (*vide infra*).

In an attempt to further improve physical chemical properties of the indazole CCR1 antagonists, heteroatoms were introduced into the core. The 5-azaindazole **18a** was 20-fold less active against CCR1 than the comparator **9e** (Table 3). However, the 6-azaindazole **18b** showed improved solubility and potency increased by an additional factor of ten (CCR1 IC_{50} 3.0 nM). Unfortunately, the solubility of the azaindazoles with the trifluoromethylbenzylamine moiety remained overall low.

It should be noted that the 7-azaindazole isomer was successfully synthesized only with additional substituents on the ring (and with using a different benzylamine moiety). With said changes, it lost all activity. The 5,7-diazaindazole **18c** was not active (CCR1

Table 4
Benzylamine SAR on the azaindazole core.



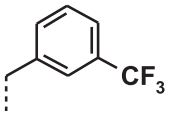
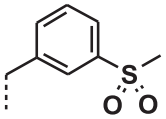
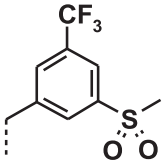
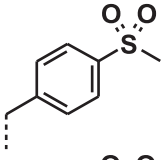
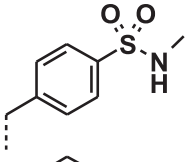
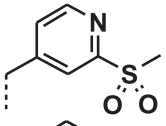
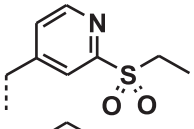
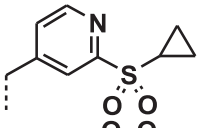
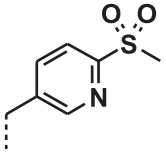
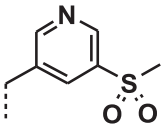
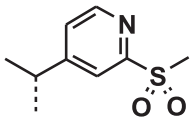
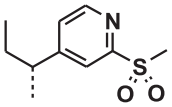
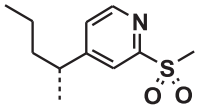
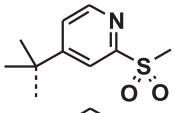
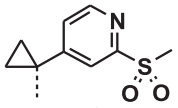
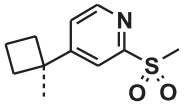
Compound	R^2	CCR1 Ca^{2+} flux IC_{50} (nM)	High-Throughput Solubility pH 7 (μg/mL)
18b		3.0	0.1
19a		8.0	18
19b		1.2	3.2
19c		12	3.5
19d		2	2.4
19e		6.8	20
19f		19	54
19 g		41	6
19 h		34	14

Table 4 (*continued*)

Compound	R^2	CCR1 Ca^{2+} flux IC_{50} (nM)	High-Throughput Solubility pH 7 (μg/mL)
19i		61	9
19j		1.4	43
19k		0.2	58
19l		0.3	48
19m		12	> 45
19n		1.8	> 45
19o		2.0	> 45

IC_{50} > 3000 nM).

It should also be noted that the potency increase of the 6-azaindazole compared to the indazole could be realized equally through the introduction of a hydrogen bond acceptor substituent (such as CN) in the 6-position of the indazole. However, the solubility of the 6-cyanoindazoles was more difficult to improve.

With nanomolar CCR1 antagonists like **18b** in hand, less potent benzylamine moieties from Table 2 were re-evaluated. The 3-methylsulfonyl compound **19a** was 2- to 3-fold less potent than **18b** but had 100-fold increased kinetic solubility (Table 4). Interestingly, the methylsulfonyl and trifluoromethyl groups appeared to bind in different orientations at the receptor, since **19b** containing both groups was one of the most potent compounds (CCR1 IC_{50} 1.2 nM). The *para*-isomer **19c** showed similar CCR1 activity (IC_{50} 12 nM) as **19a** but lower aqueous solubility. The corresponding *N*-methylsulfonamide **19d** was slightly more potent. These trends confirmed data found in the indazoles (see Table 2). However, when the sulfonyl substitution was combined with introducing a pyridyl nitrogen atom, in this series, the potency did not diminish. Methylsulfonyl pyridine **19e** was equipotent to **19a**. Analysis of crystalline thermodynamic solubility (see also advanced profiling, *vide infra*) revealed that the additional nitrogen atom improved solubility further. With a calculated logP of 2.0 and 91 $Å^3$ polar surface area, this compound was expected to show excellent drug-like properties. Increasing the size of the alkyl group in the sulfone substituent to ethyl and cyclopropyl as in **19f** and **19g**, respectively, led to a slight reduction in potency. The two regioisomer analogues of **19e**, **19h** and **19i**, keeping the sulfone substituent and the pyridine nitrogen atom in the preferred *para*- and *meta*-positions, showed a reduction of potency too. Additional possible regioisomers were less active.

Table 5
Profile of select compounds.

Compound	CCR1 Ca^{2+} flux IC_{50} (nM)	CCR1 Chemotaxis IC_{50} (nM)[a]	HLM[b] CL (%Q_h)	CYP3A4 IC_{50} (μM)	HT Solubility pH 7 (μg/mL)	hERG IC_{50} (μM)[c]	MW	clogP
19e	6.8	28	34	> 30	20	30	425	2.0
(*S*)-19k	0.2	0.3	18	17	> 45	> 30	454	2.9
19n	2.0	2.0	< 30	> 30	> 45	68	452	2.9

NT, not tested.
[a] CCR1chemotaxis in THP-1 cells[35].
[b] Human liver microsomes (HLM).
[c] Manual patch clamp system.

Lastly, the discovery was made that α-branching in the benzylic position with small alkyl groups could significantly increase the potency in the related pyrazole series (see immediately preceding publication in this issue). This concept was explored with the azaindazole core. The indazoles **13** derived from racemic 1-(3-trifluoromethylphenyl)ethylamine (CCR1 IC_{50} 350 nM) and the commercially available (*S*) and (*R*) enantiomer of 1-(3-trifluoromethylphenyl) ethylamine to afford both single enantiomers was synthesized. This confirmed that the *S*-enantiomer was the optimal stereochemistry for further SAR studies with respect to substitution in benzylic position ((*S*)-enantiomer IC_{50} 350 nM and (*R*)-enantiomer IC_{50} > 3000 nM in the calcium flux assay).[29] The racemic compounds **19j-l** bearing a methyl, ethyl and *n*-propyl group on the benzylic position showed approximately a 5-, 30- and 20-fold further increase in potency, respectively. Compounds **19k** and **19l** showed sub-nanomolar CCR1 IC_{50} values of 0.2 and 0.3 nM, respectively. Despite the added lipophilicity, the kinetic solubility of these compounds remained high, however, as expected, crystalline material showed lower thermodynamic solubility (see advanced compound profiles). Finally, *gem*-dimethyl substitution as in **19m** was not preferred, however, the cyclized cyclopropylidine and cyclobutylidine analogues **19n** and **19o** did not lose potency (IC_{50} 1.8 nM and 2.0 nM, respectively) compared to the methyl analogues **19j** – simultaneously avoiding chirality and thus the need for an asymmetric synthesis.

More than 100 combination molecules in this small chemical space (variation of sulfonyl substituent and position on pyridine isomers, combined with small mono- or di-substitution on the benzylic position) were prepared and evaluated. The most promising candidates were resynthesized and, if required, resolved into pure enantiomers.

Table 5 shows advanced profiling data of three preferred candidates.[33] In general, the potency in this series in the calcium flux assay was increased over 1000-fold compared to the lead compound **9a**, with only a modest increase in MW (414 vs 454 g/mole), while reducing the overall lipophilicity of the series (4.0 vs 2.9 calc logP). In general, as expected, compounds with lower clogP displayed improved aqueous solubility. For this series, the CYP3A4 inhibition, HLM stability and hERG inhibition parameters were typically acceptable.

Methylsulfone **19e** was the first of the preferred compounds identified. It was selected for full pre-clinical candidate profiling: The functional potency in combination with moderately low plasma protein binding (74%) translated well into human whole blood activity (receptor internalization (RI) assay; IC_{50} 47 nM; mean of 8 donors).[34] The compound was approximately 100-fold less potent against rodent CCR1 (mouse CCR1 IC_{50} 670 nM) and was therefore not profiled in any *in vivo* rodent models of inflammation. The metabolic stability was confirmed as high (9%Q_h in hepatocytes). This translated into an attractive pre-clinical PK profile: The compound showed low clearance of 8%Q_h, V_{SS} 1.2 l/kg, and moderate bioavailability of 29% in a rat PK study (Sprague-Dawley rat, po at 10 mg/kg in 0.5% methyl cellulose/0.015% tween 80 in water; iv at 1 mg/kg in 70% PEG400 in water). Thermodynamic solubility data of crystalline material confirmed acceptable solubility of 24 μg/mL at pH 7. Based on human whole blood potency (and the conservative assumption of a requirement of > 90% receptor coverage at trough), the pre-clinical PK properties, *in vitro* human clearance data and predicted bioavailability, a human efficacious dose of 15 mg *qd* was predicted. Safety profiling showed acceptable safety margins in acute and chronic, rodent and non-rodent toxicity studies. The compound was advance into early clinical trials. Clinical data will be published in a future publication.

Compound **(*S*)-19k** showed 30-fold further improved potency. It inhibited CCR1 RI in human whole blood with an IC_{50} of 1 nM (n = 6 donors). This represents the most potent CCR1 antagonist from any internal or published series tested in the in-house assay, including competitor's clinical candidates. Compound **(*S*)-19k** was indeed so potent that despite a 200-fold reduced potency in rodent (mouse CCR1 IC_{50} 44 nM) the compound was suitable to be profiled in a mouse collagen antibody induced arthritis (CAIA) mouse model: Near maximal inhibition (94%) of disease severity score was seen at day 14 with 100 mg/kg *bid* **(*S*)-19k**, approximately 50% efficacy was seen with *bid* 30 mg/kg. At 100 mg/kg *bid* **(*S*)-19k**, trough exposure 5 to 10-fold above the (mouse) whole blood cellular IC_{50} values was obtained. Exposures in mice treated with 30 mg/kg indicated compound plasma concentration at or near the whole blood IC_{50}. Histopathology results demonstrated significant improvement in the fore paw histology score, which correlated with disease efficacy as measured by the arthritic score. These results confirmed the principle of CCR1 antagonism as a key therapeutic target in this chronic model of arthritis and also underscored the need for sufficient coverage to achieve efficacy. Metabolic stability was confirmed to be low to moderate (hepatocyte CL 22%Q_h). The most stable polymorph showed thermodynamic solubility of 20 μg/mL at pH 7. The human dose for this compound was projected at 0.5–1.25 mg *qd*. Unfortunately, during pre-clinical safety testing it was discovered that the compound caused dramatic bone marrow ablation in dogs with no therapeutic margin and development was terminated. In the 4-week dog study, *ex vivo* inhibition of neutrophil CCR-1 receptor internalization was maximal 1 mg/kg/day. Sudden and rapid decreases in peripheral leukocytes, with no changes to *T*-cells, were observed by day 8 at doses of 5 but not 1 mg/kg/day resulting in early euthanasia of most dogs. These effects reversed in a 5-day survivor. Histopathology included bone marrow hematopoietic cell depletion and lymphoid depletion most severely in the thymus. Although not clastogenic, *in vitro* human lymphocyte chromosome aberration (HLA) test, **(*S*)-19k** induced a dose dependent increase in centromeric disruption and low incidence of endoreduplication. **(*S*)-19k** was positive in an *in vivo* micronucleus assay and an *in vitro* micronucleus test in CHO cells where anti-kinetochore staining confirmed aneugenicity. Although the cause of toxicity remained undetermined, primary toxicity to the hematopoietic and lymphoreticular systems could be related to aneugenic activity of **(*S*)-19k**.

Cyclopropane **19n** was advanced into pre-clinical development as a third azaindazole series compound.[36] The compound provided a back-up to **19e**. It was about 5–10-fold more potent than **19e** (Chemotaxis IC_{50} 2.0 nM; RI IC_{50} 10 nM (n = 3 donors)), it did not show the toxicology findings of **(*S*)-19k,** and it retained excellent drug-like properties: Hepatocyte stability was further improved with a clearance of < 8%Q_h; crystalline solubility at pH 7 was slightly lower with 7 μg/mL. In a rat PK study, the compound showed low clearance of 15%Q_h, V_{SS} 1.0 l/kg, and moderate bioavailability of 20% (Sprague-Dawley rat,

po at 3 mg/kg in 0.5% methyl cellulose/0.015% tween 80 in water; iv at 1 mg/kg in 70% PEG400 in water). The human efficacious dose was predicted to be 55 mg *qd*, ensuring once-a-day dosing with one dosing unit. Clinical testing of the compound was halted before initiation of Phase I due to strategic reasons. The compound is now being offered to the scientific community for basic chemokine receptor research on the Boehringer Ingelheim Open Innovation Portal OpnMe [www.opnme.com] as one of the most selective and potent CCR1 antagonist in the field.

In summary, an (aza)indazole series of CCR1 antagonists has been designed starting from a pyrazole core discovered in a HTS screen. The (aza)indazole series presented in this report represents a novel class of CCR1 antagonists which are structurally distinct from compounds that have previously entered clinical trials. Medicinal chemistry optimization in the indazole series afforded compounds with excellent human pharmacological potency and promising drug like features. The leading three compounds were advanced into pre-clinical development, with **19e** advancing in Phase I clinical trials.

Competing interest statement

The authors have no competing interests to declare.

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28. Compounds are assessed for the ability to block the interaction of CCR1 and its ligand in a functional cellular assay measuring calcium flux in response to MIP-1α in CCR1-transfected cells. Published CCR1 reference compounds gave the following IC_{50} values in this assay: BX-471 (**1**): 1.3 nM; CP-481715 (**2**): 59 nM; MLN-3897 (**3**): 0.8 nM; CCX354 (**4**): 2.0 nM; BMS-817399 (**5**): 3.0 nM. Method A: Non-adherent cells purchased from Chemicon Corporation (HTS005C), stably expressing recombinant CCR1 and G-alpha-16 are grown in RPMI 1640 medium (Mediatech 10-080-CM) supplemented with 10% heat-inactivated FBS, 0.4 mg/mL Geneticin and penicillin/streptomycin. On the day of the assay, the cells are transferred to a beaker and dye-loaded in bulk using a Fluo-4 NW Calcium Assay Kit with probenecid (Invitrogen F36205) at 0.8E6 cells/mL for 1 hour at room temperature. After 1 hour, they are seeded in a 384-well tissue culture-treated plate at a density of 20,000 cells/well. Appropriately diluted test compound is added to the well to achieve a top concentration of 3,000 nM (diluted 3-fold with 10 doses total). The final concentration of DMSO is 1%. The buffer is HBSS (Invitrogen 14025) with 20 mM HEPES at pH 7.4. The cells are allowed to incubate 1 hour in the dark at room temperature. The plates are transferred to the FLIPR TETRA where MIP-1 alpha in 1% BSA is added at the EC80 final concentration. Wells +/- MIP-1 alpha containing diluted DMSO instead of compound serve as the controls. Intracellular calcium flux is recorded on the FLIPR TETRA, using excitation at 470/495 nm and emission at 515/575 nm. Data are analyzed using Activity Base software. Method B: Non-adherent cells purchased from Chemicon Corporation (HTS005C), stably expressing recombinant CCR1 and G-alpha-16 are grown in RPMI 1640 medium (Mediatech 10-080-CM) supplemented with 10% FBS, 0.4 mg/mL Geneticin and penicillin/streptomycin. On the day of the assay, the cells are loaded with Calcium 4 dye (Molecular Devices R7448) with Probenecid (Invitrogen P346400) at 8E5 cells/mL for 1 hour at room temperature. After 1 hour, they are seeded in a 384-well tissue culture-treated plate at a density of 20,000 cells/well. Appropriately diluted test compound is added to the well to achieve a top concentration of 3,000 nM (diluted 4-fold with 10 doses total). The final concentration of DMSO is 1%. The buffer is HBSS (Invitrogen 14025) with 20 mM HEPES at pH 7.4. The cells incubate 30 minutes at 37 C and then 30 minutes at room temperature. The plates are transferred to the HAMAMATSU FDSS6000 where MIP-1alpha in 1% BSA is added at the EC80 final concentration. All plates must be read within 4 hours of the start of dye-loading. Wells +/- MIP-1alpha containing diluted DMSO instead of compound serve as the controls. Data are analyzed using Activity Base software.
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33. Analytical data for select compounds: **19e**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.62 (t, J = 6 Hz, 1H), 9.42 (s, 1H), 8.92 (s, 1H), 8.75 (d, J = 5 Hz, 1H), 8.73 (s, 1H), 8.07 (s, 1H), 7.90-7.95 (m, 2H), 7.76 (dd, J = 5 Hz, J = 1 Hz, 1H), 7.47-7.52 (m, 2H), 4.75 (d, J = 6 Hz, 2H), 3.30 (s, 3H). ES-MS m/z = 426 (M + H)$^+$. HPLC rt = 12.77 min, > 99%. **19k**: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.41 (s, 1H), 9.39 (d, J = 8 Hz, 1H), 8.95 (s, 1H), 8.76 (d, J = 5 Hz, 1H), 8.64 (d, J = 1 Hz, 1H), 8.16 (s, 1H), 7.89-7.94 (m, 2H), 7.82 (dd, J = 5 Hz, J = 2 Hz, 1H), 7.45-7.51 (m, 2H), 5.17 (dd, J = 14 Hz, J = 8 Hz, 1H), 3.32 (s, 3H), 2.50 (m, 1.85-1.99, 2H), 1.00 (t, J = 7 Hz, 3H). ES-MS m/z = 454 (M + H)$^+$. HPLC rt = 13.91 min, 98.7%. **19n:** ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.74 (s, 1H), 9.42 (s, 1H), 8.94 (s, 1H), 8.69 (d, J = 1 Hz, 1H), 8.67 (d, J = 5 Hz, 1H), 7.90-7.95 (m, 2H), 7.81 (d, J = 1 Hz, 1H), 7.46-7.54 (m, 3H), 5.17 (dd, J = 14 Hz, J = 8 Hz, 1H), 3.28 (s, 3H), 1.60 (s, 4H). ES-MS m/z = 452 (M + H)$^+$. HPLC rt = 13.55 min, 99.3%.
34. Compounds are assessed for the ability to block the internalization of CCR1 in fresh human whole blood: Compound dilutions are prepared in DMSO and in room temperature Hanks buffered saline solution, +Mg, +Ca (Gibco 14025) with 20 mM Hepes (Gibco 15630), pH 7.4, 0.2% BSA (Sigma 2058-25G). Pipetted to mix between

each dilution. Added 10 µl of 10X compound dilution or DMSO to 80 µl blood in bullet tubes (BioRad 598281) and pipetted to mix 4-6 times. Mixed compound and blood again with pipette set to 80 µl to insure total incorporation. Incubated for 30 min at 37°C. Prepared 10X MIP1α/Alexa 647; Almac Sciences; 100 µg/ml (10 µM) in PBS/0.1% BSA. Added 10 µl of MIP-1a mixtures or RI buffer to appropriate tubes at appropriate times. Mixed with pipette set to 80 µl to insure total ligand incorporation and through mixing. Added 10 µl of the 50 nM Alexa solution to compensation tubes. Mixed gently and incubated at 37°C for 40 min. Stopped reaction by putting plate on ice and addition of 0.6 mL lysing buffer per well (R&D Whole Blood Erythrocyte Lysing Kit Cat WL1000, Lot BB018042, exp 4/10 (dilute 1:10 in sterile distilled H_2O)). Mixed by pipetting up and down 300 µl ~8 times. Incubated at 4°C for 20 minutes to lyse. Centrifuged (1300 rpm, 4°C, 5 min) then removed supernatant with multi-channel pipettor (400-500 µl). Repeated lysis with 600 µl fresh lysis buffer; pipetted 6-8X. Incubated 20 min depending on first lysis efficiency. Centrifuged, aspirated and washed with 600 µl PBS/Azide (PBS (Gibco 14040) + 3% FCS (HI) (Gibco 16140-071) + 0.1% sodium azide (Fisher S-227)). Centrifuged, aspirated and washed with 600 µl acid treatment to remove surface bound ligand. Centrifuged, aspirated and washed with 600 µl PBS/Azide. Resuspended in 50 µl PBS +10% human serum buffer. Mixed 3-4 times; Incubated 30 min at 4°C. Added 50 µl of either the isotype or antibody in staining solution. Mixed 3-4X with addition. Incubated an additional 30 min at 4°C. Added PBS/5% human serum to 600 µl. Centrifuged, aspirated and washed with 600 µl PBS/Azide. Fixed the samples in 200 µl 1% formaldehyde for 10 min room temp. Transferred to FACS plate (BD Cat#353918) and stored over night at 4°C in dark. Read on LSRII

35. Compounds are assessed for the ability to block the chemotaxis of THP-1 cells. Published CCR1 reference compounds gave the following IC_{50} values in this assay: BX-471 (**1**): 1.1 nM; CP-481715 (**2**): 39 nM; MLN-3897 (**3**): 1.8 nM; CCX354 (**4**): 4.0 nM; BMS-817399 (**5**): 1.5 nM. THP-1 cells (500,000 cells/well, each plate will use 60 µl of cells per well at 1.1 x 10^7 cells/mL. Washed cells 3 times in assay buffer (HBSS + 10 mM HEPES pH 7.4 + 0.1% BSA (Sigma #A7030)) are added to media (Cell Gro #10-040-CM) add *L*-Glutamin (Cell-Gro # 25-005-CI (final 2 mM), Pen/strep (Cell-Gro # 30-002-CI) final 100 Units & FBS (Cell-Gro #35-015-CV) final 10%) in T175 flask and grown in 37°C incubator with 5% CO_2. Cells grown between 2.0 x 10^5 and 1.0 x 10^6 cell/mL. Resuspended cells to 1.1 x 10^7 cells/mL. Compounds received as dry powder are dissolved in 100% DMSO (Dri-solve EMD #MX 1457-6) at 10 mM or as liquid in 15 µl tubes (10 mM). Added reagents by using a multidrop with a disposable head: 90 µl assay buffer to 96 well plate (Falcon 35-1190), 60 µl cell suspension to a 96 well plate (Falcon 35-1190), 280 µl of assay buffer to column 12 of chemotaxis chamber, 280 µl of 6.75 nM RANTES to columns 1-11 of chemotaxis chamber. Transferred 10 µl compound titration (100% DMSO) into 90 µl assay buffer mix 6 times. Transferred 6.7 µl diluted compound to 60 µl cells in Falcon 96 well plate. Cover using lids (Falcon 35-1191), mixed by shaking. Incubated cells for 30 minutes at 37°C and 5% CO_2. Changed tips and transferred 32 µl diluted compound to lower chamber containing 280 µL 6.75 nM RANTES (R&D systems #278-RN/CF). Placed membrane on lower chamber. After 30 min incubation, mixed cells on shaker. On the Cybio added 50 µl cells to top of membrane. Incubated 3 hours at 37°C and 5% CO_2. In a 96 well (Falcon 35-1190) added 260 µl assay buffer to column 1 row A-D and 100 µl to columns 2-8 in Rows A-D. Added 50 µl cell suspension to column 1, rows A-D. Titrate 1:2 to column 7. Added lid (Falcon 35-1191) place in incubator for 3 hours. On Cybio mixed % chemotaxis plate and added 40 µl to white half area plate containing 40 µl Promega Cell Titer-Glo reagent (#G-7572). On cybio; mixed lower chamber and added 40 µl to white half area plate containing 40 µl Promega Cell Titer-Glo reagent (#G-7572). Mixed on shaker. Incubated at room temperature for 15 min. Read luminescence on LJL Analyst. % Chemotaxis generated by excel template.

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