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Piperazinyl CCR1 antagonists—optimization of human liver microsome stability

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Abstract—The synthesis, biological activity, and pharmacokinetic profile of CCR1 antagonists are described.

A number of published studies have demonstrated the potential utility of CCR1 antagonists for the treatment of human diseases including autoimmune disorders and organ transplant rejection.[1,2] We recently described medicinal chemistry efforts that led to the identification of the novel CCR1 antagonist CP-481,715 (**1**, see Scheme 1).[3–5] A Phase I study conducted with CP-481,715 (**1**) in rheumatoid arthritis (RA) patients provided the first clinical evidence that blockade of CCR1 signaling could be a viable treatment for RA as a significant reduction of monocyte infiltration into synovial tissue was observed following 2 weeks of dosing (300 mg TID × 14 days).[6] However, these positive findings were tempered by a subsequent Phase II clinical trial wherein CP-481,715 (**1**) failed to demonstrate clinical efficacy in RA patients following 6 weeks of treatment.[1] In addition, efficacy was not observed in a Phase II clinical trial wherein patients with relapsing remitting multiple sclerosis (RRMS) were administered the Berlex/Schering AG CCR1 antagonist BX471 (**2**) (600 mg TID × 16 weeks).[7] In spite of these clinical setbacks, CCR1 antagonism remains to be an attractive approach for the treatment of a number of other human diseases. Herein, we describe efforts to identify new CCR1 antagonists suitable for clinical evaluation.

A number of in-house and literature lead series were evaluated for potential follow-up. The piperazine series exemplified by BX471 (**2**), appeared to have the greatest potential to produce compounds with the desired attributes. BX471 (**2**) was reported to have excellent intrinsic potency and was shown to be effective in a variety of disease models while displaying little off-target activity.[8] Published pharmacokinetic studies conducted with BX471 in dogs described good oral bioavailability (~60%) and a moderate half-life of approximately 3 h.[8] A human half-life of approximately 2.3 h has been reported as well.[9] We evaluated the in vitro metabolic stability of BX471 (**2**) in liver microsome preparations from a number of species, including rat, dog, monkey, and human, to provide an understanding of metabolism across species (see Table 1).[10] Moderate to rapid metabolism was observed in all species, with the greatest stability being observed in dog liver microsomes (DLM). Metabolite identification studies conducted with BX471 (**2**) following incubation in human liver microsomes (HLM) suggested that N-debenzylation contributed significantly to the moderate turnover observed. The following describes our efforts to identify potent, selective, piperazine-based CCR1 antagonists with enhanced metabolic stability.

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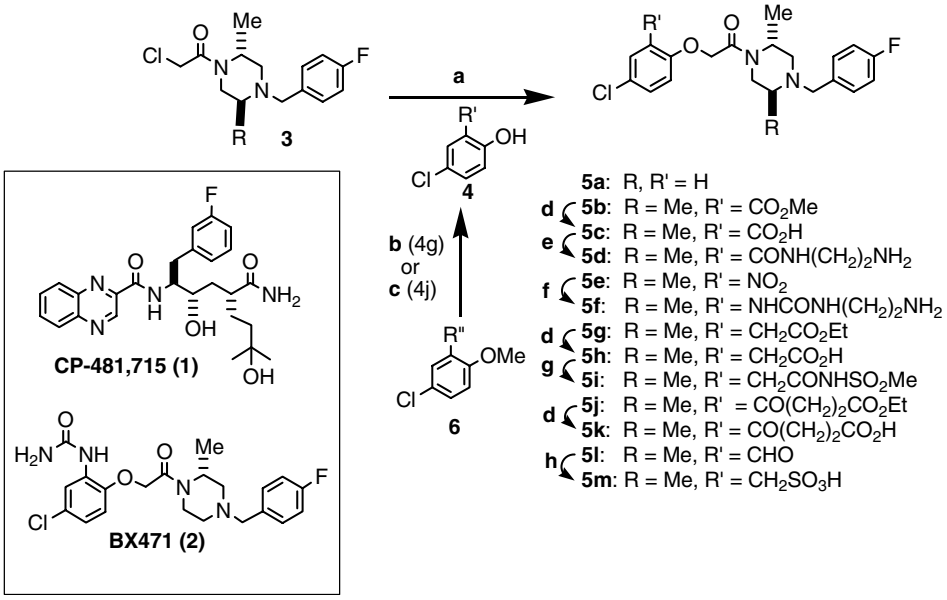

Scheme 1. Reagents and conditions: (a) 2-butanone, K_2CO_3, KI, reflux; (b) R″ = CO_2Et, (1) LAH, THF, 0 °C → reflux, (2) $SOCl_2$, CH_2Cl_2, (3) KCN, 18-C-6, CH_3CN, rt, (4) KOH, H_2O, reflux, (5) 48% HBr, reflux, (6) EtOH, HCl, rt; (c) R″ = H, (1) succinic anhydride, $AlCl_3$, DCE, rt, (2) EtOH, HCl, rt; (d) THF, methanol, H_2O, LiOH hydrate, rt; (e) BOP, Hunig's base, ethylenediamine, DMF; (f) (1) 5% PtO_2 on carbon, 35 psi H_2, EtOH, rt, (2) 4-nitrophenylchloroformate, pyridine, CH_2Cl_2, rt, (3) ethylenediamine, MeOH, rt; (g) NH_2SO_2Me, EDCI, DMAP, NEt_3, CH_2Cl_2; (h) (1) $NaBH_4$, MeOH, reflux, (2) $SOCl_2$, CH_2Cl_2, (3) Na_2SO_3, EtOH, H_2O, reflux.

Compounds were prepared in a straightforward fashion as described in Scheme 1. Phenols (**4**) were either commercially available or prepared as described, then coupled with α-chloroacetamide **3**[11] to provide analogs **5a**, **5b**, **5e**, **5g**, **5j**, and **5l**. Further elaboration provided analogs **5c**, **5d**, **5f**, **5h**, **5i**, **5k**, and **5m**.

Two general strategies were employed to improve metabolic stability. One relied on incorporating structural changes to block metabolism, the other focused on reducing molecular lipophilicity, and the latter approach is the focus of discussion for this letter. Our strategy was as follows: (1) determine the minimum pharmacophore for the series (lowest molecular weight analog which maintains significant intrinsic potency), (2) determine which position(s) of the molecule are most amenable to incorporation of polar functionality to drive down molecular Clog *D*,[12] and (3) evaluate the impact of reduced Clog *D* on metabolic stability, both in vitro and in vivo. Compound **5a** was settled on as the minimum pharmacophore as removal of either the 4-F, 4-Cl or 2-piperazinyl methyl groups led to a substantial loss of potency in binding and/or functional chemotaxis assays.[13,14] The 2-phenoxy vector was determined to be an appropriate area to explore with new analog generation as BX471 (**2**) incorporates a polar urea in this position. Indeed, a wide variety of neutral, basic and acidic substituents could be incorporated at this position without sacrificing intrinsic potency. While neutral polar groups (e.g., amides, sulfonamides, ureas, carbamates, etc.) maintained acceptable potency, a variety of ADME issues plagued this set, including poor microsome stability and/or intestinal efflux.[15] Therefore, analogs incorporating neutral 2-phenoxy substituents were not extensively pursued.

Incorporation of 2-phenoxy groups containing basic amines efficiently reduced Clog *D* values relative to BX471 (**2**), which generally translated to improved microsomal stability. For example, the amino analogs **5d** and **5f**, which were potent in binding, functional and human whole blood assays[16] were found to be reasonably stable in liver microsomes across a number of species. Unfortunately, the improved microsomal stability did not translate in vivo as **5d** and related analogs were found to undergo rapid clearance in rats following iv dosing. The reason for the rapid plasma clearance was not discerned, however, it may have been the result of non-CYP450 mediated metabolism, drug transporter-mediated clearance, and/or partitioning into red blood cells. Compound **5d** was also evaluated for selectivity against a panel of receptors. While BX471 (**2**) has been reported to exhibit excellent selectivity for the CCR1 receptor,[8] **5d** showed measurable activity against several targets (adrenergic, dopaminergic, and calcium channel) suggesting that the incorporation of an additional basic moiety had led to a significant erosion of selectivity.[15]

Zwitterionic compounds derived from incorporation of acidic groups at the 2-phenoxy position were explored as well. Benzoic acid **5c** displayed moderate potency in the receptor binding and chemotaxis assays. However, whole blood activity was poor (>10 μM), most likely due to extensive binding to plasma proteins (<1% unbound). As was the case for the amines described above, incorporation of an acidic moiety significantly lowered the Clog *D* leading, in general, to improved microsomal stability. However, unlike the amines, zwitterion **5c** displayed much improved pharmacokinetic behavior in vivo, prompting us to focus our efforts on identifying zwitterions with acceptable whole blood activity.

Table 1. Potency data and pharmacokinetic study results

Compound	CCL3 binding IC_{50} (μM)[13]	CCL3 chemotaxis IC_{50} (μM)[14]	Human whole blood IC_{50} (μM)[16]	[a]HLM RLM DLM MLM Cl_b^{10}	Clog *D* at pH 7.4[12]	Species	%*F*	Clp[b]	Vdss (L/kg)	$T_{1/2}$ (h)
1	0.048	0.066	0.22	5.0 38 11 20	1.9	Dog Monkey	48 10	13 17	1.2 1.1	1.5 0.9
2	0.031	0.004	0.062	16 62 28 42	2.8	Rat Dog Monkey	2 NT NT	33 1.7 8.9	1.7 0.6 0.6	0.7 4.0 0.8
5a	0.086	0.086	NT	14 63 27 40	3.7	NT	NT	NT	NT	NT
5c	0.059	0.068	> 10.00	4.7 20 11 31	0.9	Rat Dog Monkey	NT NT NT	2.4 0.2 3.4	2.4 0.2 0.9	14 11 5.9
5d	0.030	0.003	0.007	5.9 22 25 36	1.0	Rat	NT	184	7.7	1.0
5f	0.008	0.001	0.011	4.9 24 11 18	1.4	NT	NT	NT	NT	NT
5h	0.038	0.002	>10.00	4.7 25 11 18	0.3	Rat Dog	NT NT	5.2 0.4	3.2 0.3	3.7 11
5i	0.044	0.002	0.321	4.7 26 11 18	0.5	Rat Dog Monkey	19 100 24	4.5 0.09 1.2	0.5 0.09 0.2	1.4 13 1.2
5k	0.013	<0.0005	0.108	4.7 31 11 18	0.9	Rat Dog	9 100	42 9.7	4.0 4.2	2.3 5.8
5m	0.023	0.003	0.39	4.7 21 11 18	−1.6	Rat Dog Monkey	40 100 19	17 0.2 51	2.6 0.4 2.1	4.6 21 1.1

NT, not tested.

[a] Human liver microsomes (HLM), rat liver microsomes (RLM), dog liver microsomes (DLM), monkey liver microsomes (MLM); units: mL/min/kg.

[b] Clp, plasma clearance (units: mL/min/kg).

Homologation of the benzoic acid **5c** provided benzylic acid **5h**. While this analog displayed similar potency to **5c** in the receptor binding assay, **5h** was approximately 30-fold more potent in the functional chemotaxis assay. Similarly, acylsulfonamide **5i**, keto acid **5k**, and sulfonic acid **5m** were all found to exhibit low nanomolar functional activity, and importantly, all demonstrated acceptable whole blood activity and HLM stability. Having identified compounds with appropriate potency and microsome stability, follow-on pharmacokinetic studies were performed. As was the case with benzoic acid **5c**, benzylic acid **5h** and acylsulfonamide **5i** demonstrated improved in vivo clearance as compared to BX471 (**2**) in the species evaluated. However, keto acid **5k** demonstrated similar rat clearance and somewhat greater dog clearance as compared to BX471 (**2**). Sulfonic acid **5m** demonstrated low plasma clearance following iv dosing in dogs and rats, and good exposure following oral dosing. However, rapid clearance was observed in monkeys for **5m**. The greater than predicted clearance observed for **5k** in dogs and rats and, **5m** in monkeys may have been due to a number of factors including phase II metabolism and/or drug transporter mediated clearance. Additionally, while amine **5d** described above demonstrated poor broad selectivity, sulfonic acid **5m** displayed excellent selectivity for the CCR1 receptor.[15]

In summary, medicinal chemistry efforts to identify piperazine analogs with improved metabolic stability were described. While analogs incorporating amino groups at the 2-phenoxy position suffered a number of issues, including poor in vivo pharmacokinetics and suboptimal broad selectivity, several zwitterionic analogs

demonstrated promising profiles. In general, compounds described herein with Clog D values $\leqslant 2$ provided in vitro human clearance values (Cl_b) at or near the lower limit of the assay utilized.[10] Additional studies conducted with sulfonic acid **5m** will be reported in due course.

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