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Pyrazolone methylamino piperidine derivatives as novel CCR3 antagonists

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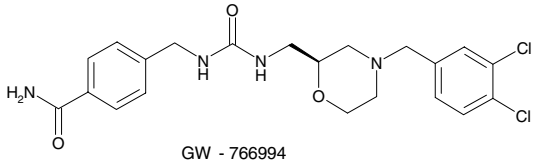
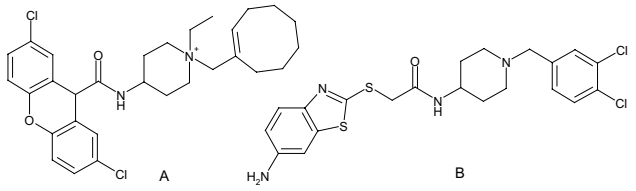
Abstract—The discovery and optimization of a novel class of potent CCR3 antagonists is described. Details of synthesis and SAR are given together with some ADME properties of selected compounds. An optimal balance between activities, physicochemical properties, and in vitro metabolic stability was reached by the proper choice of substituents.

The prevalence of asthma is increasing in industrialized countries and symptoms are not completely controlled in many patients. There is a need for new therapies against this chronic airway inflammatory disease. Pulmonary eosinophil recruitment seems to be closely related to the symptoms of allergic asthma. Eosinophils are attracted, via their CC chemokine receptor 3 (CCR3), in response to chemoattractants such as eotaxin, Rantes, MCP-3, MCP-4 released in the airways of asthmatics.[1] Inhibiting pulmonary eosinophilia by blocking the CCR3 receptor with small molecule antagonists may lead to a reduction in the inflammation and the airway responsiveness seen in asthma. Several research groups are investigating this approach.[2] One compound from GlaxoSmithKline (GW-766994)[3] is currently undergoing Phase II clinical trials for the treatment of asthma and allergic rhinitis.



GW - 766994

Among the first CCR3 antagonists described in the literature, two compounds with high affinities were disclosed by Banyu Pharmaceuticals (**A**: $IC_{50} = 0.58$ nM and **B**: $IC_{50} = 2.3$ nM).[4] We initiated a library synthesis with an aminopiperidine central core as a privileged structure to explore the chemical space and find new ligands for the CCR3 receptor. A large combinatorial library was designed around three points of diversity. The selection of the building blocks was based on different criteria like commercial availability of the reagents, physicochemical properties and diversity requirements of the generated virtual library. A 2888-compound library was prepared by solid-phase chemistry following Scheme 1. A Wang resin was activated by reaction with *p*-nitrophenylchloroformate in order to allow the attachment of 4-piperidone. The keto function was transformed after two consecutive reductive amination reactions, first with primary amines (R^2NH_2) then with aldehydes (R^1CHO) to afford resin-bound 4-dialkylamino piperidines. Cleavage from the resin using trifluoroacetic acid yielded piperidines that were finally reacted with acyl chlorides (R^3COCl).



Very few hits were identified from this large library, compound **1** being the most potent for the human CCR3 receptor ($IC_{50} = 158$ nM),[5] all bearing a phenyl pyrazolone moiety as R^1 modulation. This subunit was previously mentioned by Gong[6] as providing some

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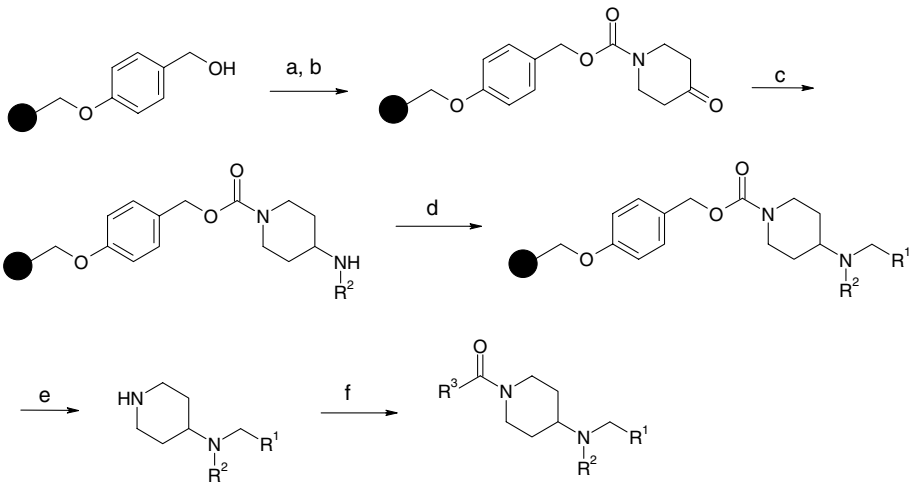
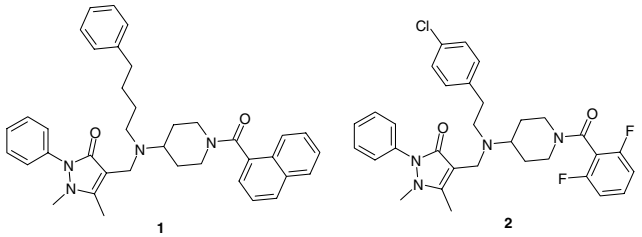
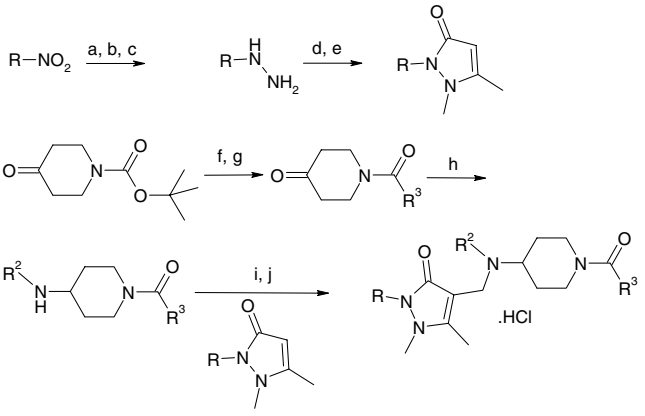


Scheme 1. Combinatorial chemistry procedure. Reagents and conditions: (a) *p*-nitrophenylchloroformate, pyridine, DCM; (b) 4-piperidone, DMF; (c) R^2NH_2, $NaBH(OAc)_3$, DCE, AcOH; (d) R^1CHO, $NaBH(OAc)_3$, DCE, AcOH; (e) TFA, DCM; (f) R^3COCl, NEt_3, DCE.

recognition for the CCR3 receptor. Further focused combichem libraries were then generated in order to optimize CCR3 binding affinity by modulating R^2 and R^3 substituents. A total of 790 compounds were prepared by the combichem procedure. The SARs revealed that the *para*-substituted phenylethyl and the *ortho*, *ortho*-disubstituted phenyl were good modulations for R^2 and R^3 moieties, respectively. In particular, compound **2** displayed a good activity for CCR3 (IC_{50} = 32 nM).





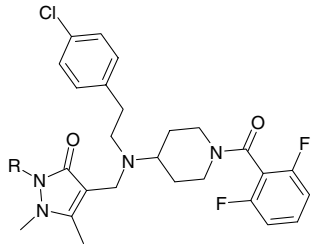
Scheme 2. Medicinal chemistry procedure. Reagents and conditions: (a) $SnCl_2$, conc. HCl, EtOH; (b) $NaNO_2$, conc. HCl; (c) $SnCl_2$, conc. HCl; (d) methyl acetoacetate, CH_3CN, reflux; (e) Me_2SO_4, CaO, MeOH; (f) TFA, DCM; (g) R^3COCl, NEt_3, DCM; (h) R^2NH_2, $NaBH(OAc)_3$, DCE, AcOH, molecular sieves; (i) paraformaldehyde, iPrOH, NH_4Cl, 90 °C; (j) methanolic HCl, ether.

We next decided to optimize the substitution pattern on the phenyl ring attached to the pyrazolone. Compounds were obtained following the route depicted in Scheme 2. Diverse pyrazolones were prepared from commercially available hydrazines by condensation with methyl acetoacetate followed by N-methylation with dimethylsulfate. Some hydrazines were prepared from nitrobenzenes by successive reduction into anilines, diazotation, and reduction by $SnCl_2$ of the diazo intermediates. The aminopiperidine core was obtained in three steps from *N*-Boc-piperidone following a Boc-deprotection, acylation, and then reductive amination sequence. The pyrazolone moiety is incorporated in the last step via a Mannich reaction.

The binding data on human CCR3 receptor[5] of compounds **2**–**18** are reported in Table 1. By comparison with the unsubstituted phenyl analogue **2**, the results show an overall decrease in affinity, except for the *para*-fluorophenyl and the 2-pyridyl derivatives. Moving a fluorine atom around the phenyl ring indicated the *meta* substitution is slightly disfavored. Most compounds display an IC_{50} around 100 nM, regardless of the presence of electron donating (OMe, NMe_2) or withdrawing (NO_2, CN) substituents. A more dramatic decrease in affinity is observed with the *para*-carboxyl analogue **14** (IC_{50} = 2500 nM) suggesting that the presence of a negative charge in this area is detrimental to the binding with the receptor.

The most potent derivative was the *para*-fluoro phenylpyrazolone analogue **5** (IC_{50} = 20 nM). Interestingly, this compound exhibits comparable level of activity in functional assays such as the inhibition of the in vitro Ca^{2+} flux and the eotaxin-induced eosinophil shape change (IC_{50} = 16 nM for both). Some physicochemical properties and in vitro metabolic clearances of compound **5** were measured and are reported in Table 2, showing poor water solubility and metabolic stability.

Table 1. CCR3 binding activities for compounds **2**–**18**



2-18

Compound	R	Binding IC_{50}[a] (nM)
2	Ph	32
3	2-Pyridyl	25
4	Cyclohexyl	100
5	4-F-Ph	20
6	3-F-Ph	100
7	2-F-Ph	40
8	4-Cl-Ph	63
9	4-OH-Ph	100
10	4-NO_2-Ph	100
11	4-CN-Ph	158
12	4-OMe-Ph	100
13	4-NH_2-Ph	79
14	4-COOH-Ph	2,500
15	4-NHCOMe-Ph	400
16	4-NMe_2-Ph	100
17	4-Cl-(3-pyridyl)	100
18	4-OMe-(3-pyridyl)	250

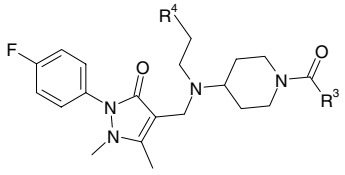
[a] Competition binding experiments with [^{125}I]eotaxin on human eosinophils.

Nevertheless, it is predicted to have good intestinal permeability according to the Caco-2 experimental model.

In order to increase solubility and metabolic stability, some less lipophilic analogues were prepared by the introduction of ionizable groups. Data from selected compounds with improved profiles are reported in Table 2. Several structural modifications generated more soluble compounds like the substitution of the chlorine atom in R^4 moiety by a fluorine (**19**), a nitro (**20**), a sulfonamido (**25**) or an amino (**26**) group (100- to 900-fold). The increase was even more significant with the replacement of one phenyl ring by a pyridine (**21**–**24**) or a pyridine N-oxide **27** (1200- to 2000-fold). Not surprisingly, the metabolic stability was concomitantly improved with those compounds, reaching acceptable levels on rat microsomes for analogues **24**–**27** (Clint < 70 μl/min/mg protein). Those results were confirmed on rat hepatocytes for compounds **24**, **26**, and **27**. On the contrary, by lowering the lipophilicity, the intestinal permeability of some analogues (**22**, **25**–**27**) could be limited, as predicted in the Caco-2 assay (Papps $< 2 \times 10^{-6}$ cm/s). Regarding CCR3 binding affinities, structural modulations in the R^4 moiety generally lead to less active analogues, whereas modifications in the R^3 part provided potent compounds (**23**, **24**, **27**). Two candidate compounds **24** and **27** will be proposed for in vivo DMPK evaluation. They both have interesting CCR3 affinities and solubility profile, **24** having good predicted intestinal permeability and moderate metabolic stability and **27** showing a better metabolic profile but a lower predicted intestinal permeability.

In conclusion, we described the discovery of a novel class of CCR3 antagonists: the pyrazolone methylamino piperidine derivatives. Lead optimization allowed the identification of potent analogues characterized by improved water solubility and metabolic stability.

Table 2. Physicochemical properties and in vitro metabolic stability of selected compounds



Compound	R^4	R^3	Binding IC_{50}[a]	Water solubility[b]	Cl_{int} on rat microsomes[c]	% parent drug consumption on rat hepatocytes after 2 h	% parent drug consumption on rat hepatocytes after 19 h	Papps[d]
5	4-Cl-Ph	2,6-F_2-Ph	20	0.001	472	52	95	8.1
19	4-F-Ph	2,6-F_2-Ph	200	0.1	303	nt	nt	nt
20	4-NO_2-Ph	2,6-F_2-Ph	6.3	0.1	235	35	95	nt
21	3-Pyridyl	2,6-F_2-Ph	2,500	>2	113	nt	nt	nt
22	2-Pyridyl	2,6-F_2-Ph	1,000	1.5	82	35	78	1.4
23	4-NO_2-Ph	2,6-$(Me)_2$-pyrid-3-yl	12	1.2	79	38	94	nt
24	4-NO_2-Ph	2-Pyridyl	12	1.26	62	29	82	7.8
25	4-SO_2NH_2-Ph	2,6-F_2-Ph	>10,000	0.4	<50	43	86	<1
26	4-NH_2Ph	2,6-F_2-Ph	2,500	0.9	<50	23	71	<1
27	4-Cl-Ph	2-Pyridyl-*N*-oxide	200	>2	<50	15	55	1.3

[a] Competition binding experiments with [^{125}I]eotaxin on human eosinophils in nM.
[b] In mg/ml at pH 7.4.
[c] In μl/min/mg protein.
[d] Apical to basolateral flux through a Caco-2 monolayer in 10^{-6} cm/s. nt, not tested.

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5. CCR3 binding assays were performed according to Kitaura et al.[7] with slight modifications. Briefly, 50,000 human eosinophils were incubated for 60 min at 25 °C in 200 μl of RPMI 1640 containing 25 mM Hepes (pH 7.4), 0.1% BSA, 0.1 nM [^{125}I]eotaxin (Amersham Biosciences, Belgium; 2000 Ci/mmol), and a test compound at the desired concentration. Final DMSO concentration in each sample is 2%. After incubation, bound and free radioligand are separated by rapid vacuum filtration through glass fiber filters (type GF/B or GF/C) presoaked for 2 h in PEI 0.3% in order to reduce nonspecific binding. Samples and filters are washed with 4×2 ml of an ice-cold 50 mM Tris HCl (pH 7.4) buffer containing 500 mM NaCl. Radioactivity trapped onto the filters is measured by liquid scintillation. Nonspecific binding is determined in the presence of 1 μM of compound **A**.
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