Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 497–500
Bioorganic & Medicinal Chemistry Letters

2-Mercaptoimidazoles, a new class of potent CCR2 antagonists

Guy Van Lommen,[a,*] Julien Doyon,[a] Erwin Coesemans,[a] Staf Boeckx,[a] Marina Cools,[b] Mieke Buntinx,[a] Bart Hermans[a] and Jean VanWauwe[a]

[a]*Inflammation Research, Johnson and Johnson Pharmaceutical Research and Development, Turnhoutseweg 30, B-2340 Beerse, Belgium*
[b]*Barrier Therapeutics, n.v. Cipalstraat, 3 B-2440 Geel, Belgium*

Received 13 October 2004; revised 18 November 2004; accepted 25 November 2004
Available online 16 December 2004

Abstract—We describe the synthesis and SAR of a new class of CCR2 antagonists based on a 2-mercaptoimidazole scaffold. The initial lead **1a** was optimized to the 3,4-disubstituted analogues **1p**-(*S*) and **1q**-(*S*), which have IC_{50} values in the MCP-1 induced Ca-flux below 0.01 μM.

1. Introduction

Monocyte chemoattractant protein-1 (MCP-1) and its receptor (CCR2) have been implicated in a number of inflammatory and autoimmune diseases,[1] in particular, by affecting monocyte infiltration. So antagonists of the CCR2 receptor are potential therapeutic agents for various pathological conditions such as rheumatoid arthritis,[2] multiple sclerosis,[3] COPD[4] and atherosclerosis.[5]

Recently a number of small molecule antagonists of this receptor have been described[6–11] and a common characteristic of their binding site was proposed.[12]

In a high throughput screening based on MCP-1 induced GTPγS binding in human CCR2B-CHO cell membranes, we identified the mercaptoimidazole **1a** as a new promising lead compound, its activity was confirmed in an assay based on MCP-1 induced calcium flux in THP-1 cells[14] IC_{50}: 0.2 μM.

Herein we describe the SAR in this new class of CCR-2 antagonists.

2. Chemistry

The mercaptoimidazoles were synthesized as described in Scheme 1. Starting ketones and amines were prepared by literature procedures, previously undescribed ketones could be obtained by Friedel–Crafts reactions of an acylchloride with the appropriate substituted benzene derivative. Enantiomers were obtained similarly using optically pure amines synthesized as described in Scheme 2.[13]

4,5-Disubstituted imidazoles can be prepared following Scheme 1 but using dimethyloxalate rather than methylformiate in step f. When chloroacetonitrile is used in step d, the corresponding 5-nitriles are obtained although in lower yields than the esters.

The 5-esters can be hydrolyzed by NaOH (1 N) at reflux and the corresponding acids were reacted with $SOCl_2$ to give the acid chlorides. Reaction of these with amines gave the 5-amides shown in Table 3. Also reduction of the 5-ester **1a** with superhydride in THF afforded the alcohol **3r** in good yield.

In the 4,5-diester series, the ester in the 4-position can be selectively hydrolyzed to the 4-acid 5-ester under mild conditions (1 N NaOH, rt), however, the 5-ester is much more resistant to hydrolysis and when the temperature is raised, decarboxylation became predominant.

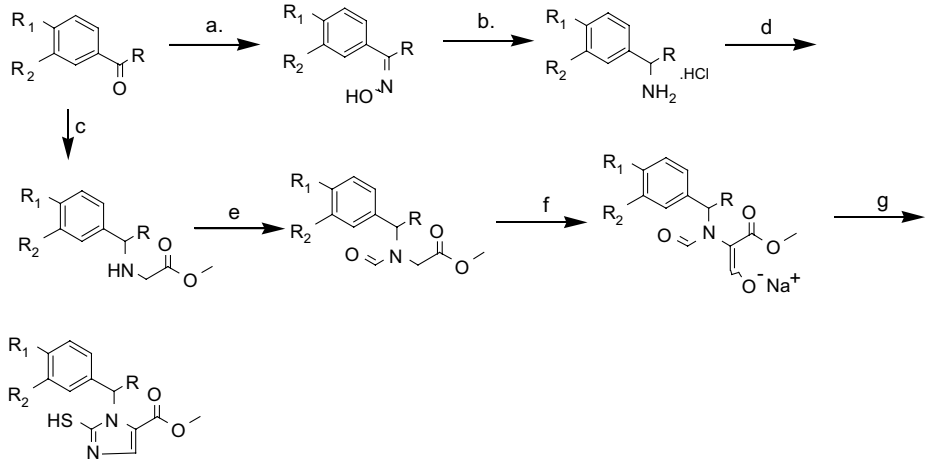
We could obtain low yields of diamides **3l** and **3m** by heating the diester **1p** in concentrated NH_4OH solution or in methanol saturated with methylamine.

3. Structure–activity relationship

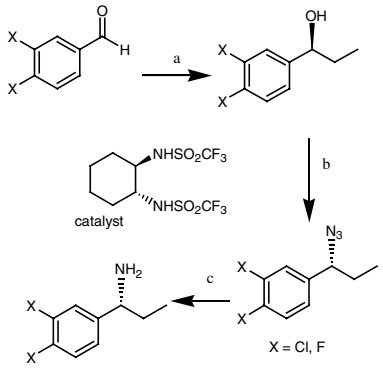
Analysis of the high throughput screening data pointed to the importance of the 3,4-dichloro substitution on the

Keywords: CCR2 antagonists; Mercaptoimidazoles.
* Corresponding author. Tel.: +32 14 603656; fax: +32 14 605344; e-mail: gvlommen@prdbe.jnj.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.11.064



Scheme 1. Synthetic route to 2-mercaptoimidazoles. Reagents and conditions (yields for R_1/R_2 = Cl): (a) $NH_2OH{\cdot}HCl$, NaOAc, MeOH [rt, 18 h] (100%); (b) hydrogenation over RaNi/thiophene in $MeOH/NH_3$ (85%); (c) methylglycinate/Pd/C 5% methanol, thiophene (80%); (d) methylbromoacetate, TEA, DMF [rt, 3 days] (80%); (e) HCOOH, xylene [reflux, 6 h] (100%); (f) HCOOMe, NaO*t*-Bu, THF [rt, 20 h]; (g) KSCN, $HCl–H_2O$, MeOH [60 °C, 20 h] (70% over f + g).



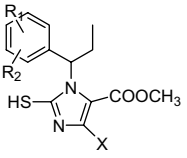
Scheme 2. Stereospecific synthesis of 1-(3,4-dihalo-phenyl)-1-aminopropane. Reagents and conditions (yields): (a) $Zn(CH_2CH_3)_2$, Ti(*i*-PropO)$_4$, catalyst, toluene, −78 °C (100%); (b) diphenylphosphorylazide, DBU, toluene (75%); (c) PdC (10%), H_2, methanol (75%).

phenyl ring. Analogues with other substituents on the phenyl (mono- or di-methyl or methoxy, and mono-halo or phenoxy) were less active. The mercapto group seemed essential for activity, desulfurized analogues were devoid of activity. Also variation in the length of the benzylic spacer led to compounds with markedly reduced potency.

To get a better idea about the SAR in this series a set of new analogues was synthesized and their biological activity was evaluated in the MCP-1 induced calcium flux assay in THP1-cells, results are summarized in Tables 1–4.

The results shown in Table 1 confirm the importance of the 3,4-dihalo (or CF_3) substitution, more importantly we also found that the introduction of a second ester in the 4-position of the imidazole substantially enhanced the activity and an IC_{50} of 10 nM or below was obtained with a number of these di-substituted compounds, in particular, **1p** and **1q**.

Table 1. Substitutions on the phenyl ring



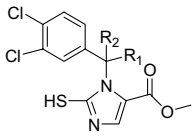
Compd	R_1	R_2	X	pIC_{50}*
1a	3-Cl	4-Cl	H	6.7
1b	H	H	H	<5
1c	2-Cl	H	H	<5
1d	3-Cl	H	H	<5
1e	4-Cl	H	H	<5
1f	4-CF_3	H	H	<5
1g	3-O-phenyl	H	H	<5
1h	3-F	4-F	H	5.9
1i	3-Br	4-Br	H	6.6
1j	3-F	4-CF_3	H	5.7
1k	3-CF_3	4-F	H	6
1l	3-OCH_3	4-OCH_3	H	<5
1m	3-CH_3	4-CH_3	H	<5
1n	3-F	5-F	H	<5
1o	3-Cl	5-Cl	H	<5
1p	3-Cl	4-Cl	$COOCH_3$	8
1q	3-F	4-F	$COOCH_3$	8
1r	3-F	4-CF_3	$COOCH_3$	7.7
1s	3-CF_3	4-F	$COOCH_3$	7
1t	3-Cl	4-Cl	CH_3	6.6

* Standard deviation <0.24.

Next we evaluated the role of the benzylic alkyl side chain. Both smaller and larger groups, acyclic and cyclic, were tolerated as shown in Table 2. An aromatic substituent at this position gave less potent compounds. Within the synthesized series the ethyl group seemed optimal.

Table 3 summarizes the results obtained with variations of the ester moieties, in particular nitriles and amides. Again compounds with 4-,5-disubstitution had the highest activity with the exception of the monoamide **3a**, that showed a surprisingly high activity.

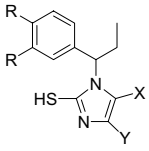
Table 2. Variations of the alkyl substituent

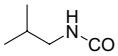
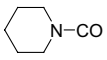
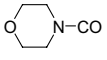


Compd	R_1	R_2	pIC_{50}*
2a	H	Methyl	6.6
2b	H	Propyl	6.8
2c	H		5.7
2d	H		6.5
2e	H	Cyclohexyl	5
2f	H	3,4-Di-Cl-phenyl	<5
2g	H	Thienyl	<5
2h	CH_3	CH_3	<5

* Standard deviation <0.15.

Table 3. Variations of the ester groups



Compd	R	X	Y	pIC_{50}*
3a	Cl	$CONH_2$	H	7.3
3b	Cl	$CONHCH_3$	H	6.5
3c	Cl	CN	H	6.2
3d	F	COOEt	H	6.5
3e	Cl	COO*i*-Prop	H	6.5
3f	F	$CONH_2$	H	6.3
3g	F	$CONHCH_3$	H	5.7
3h	F	CONBut	H	<5
3i	F		H	<5
3j	F		H	<5
3k	Cl	$COOCH_3$	COOH	<5
3l	Cl	$CONHCH_3$	$CONHCH_3$	6.4
3m	Cl	$CONH_2$	$CONH_2$	7.2
3n	Cl	CN	$CONH_2$	6.9
3o	Cl	CN	$COOCH_3$	7.4
3p	Cl	$CONHCH_3$	CH_3	<5
3q	Cl	COOH	H	<5
3r	Cl	CH_2OH	H	<5
3s	F		H	<5
3t	F		H	<5

* Standard deviation <0.40.

Alkylated amides, the acids, both in 4- and 5-position (**3q** and **3k**) and the alcohol **3r** were less active than the corresponding primary-amides and esters.

Table 4. Stereospecificity of the activity

Compd	pIC_{50}*
1a Racemic	6.7
S	6.9
R	5.8
1p Racemic	8.0
S	8.1
1q Racemic	8.0
S	8.1
R	5.7
3a Racemic	7.3
S	7.4
R	5

* Standard deviation <0.24.

Finally we investigated the stereo specificity of the CCR2 antagonistic activity and the *S*-isomers were found to be active whereas the *R*-isomers showed only weak activity as shown in Table 4. Again the monoamide **3a**-(*S*) was very active, whilst the *S*-isomers of the di-esters **1p**-(*S*) and **1q**-(*S*) were the most potent compounds obtained within this series.

These three compounds were evaluated for their effects on MCP-1 induced chemotaxis in human monocytes.[15] The amide **3a**-(*S*) has only a moderate activity (pIC_{50} 1 μM), more in agreement with the expected activity of monosubstituted compounds, but the two di-esters **1p**-(*S*) and **1q**-(*S*) were also highly active in this test with an IC_{50} value below 0.1 μM.

The effect of **3a**-(*S*) and **1p**-(*S*) on Ca-flux induced by stimulation of other chemokine receptors was evaluated. They showed a selectivity of >100 for the effect seen with CCR2 stimulation versus the effects seen with stimulation of CCR1, CCR3, CCR5, CXCR1, CXCR3 and CXCR4 receptors.

In conclusion, we have identified a new class of CCR2 receptor antagonists, structurally unrelated to other described classes. In particular, **1p**-(*S*) and **1q**-(*S*) were potent and selective antagonists and showed good functional activity. Further modifications of these structures and their in vivo activity will be the subject of future publications.

References and notes

1. Dawson, J.; Miltz, W.; Mir, A.; Wiessner, C. *Expert Opin. Ther. Targets* **2003**, *7*(1), 35–48.
2. Gongh, J. H.; Ratkay, L. G.; Waterfield, J. D.; Clark-Lewis, I. *J. Exp. Med.* **1997**, *186*, 131–137.
3. Izikson, L.; Klein, R. S.; Luster, A. D.; Weiner, H. L. *Clin. Immunol.* **2002**, *103*(2), 125–131.
4. Donnelly, L. E.; Duncan, F. R. *Drugs* **2003**, *63*(19), 1973–1998.
5. (a) Leonard, E. J. *Challenges Mod. Med.* **1994**, *3*, 25; (b) Ross, R. *New Engl. J. Med.* **1999**, *147*, 213–215.
6. Forbes, I. T.; Cooper, D. G.; Dodds, E. K.; Hickey, B.; Ife, R. J.; Meeson, M.; Stockley, M.; Berkhout, T. A.; Johil, J.; Groot, P. H. E.; Moores, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1803–1806.

7. Mirzadegan, T.; Diehl, F.; Ebi, B.; Bhakta, S.; Polsky, I.; McCarley, D.; Mulkins, M.; Weatherhead, G. S.; Lapierre, J. M.; Dankwardt, J.; Morgans, D.; Wilhelm, R.; Jarnagin, K. *J. Biol. Chem.* **2000**, *275*, 25562–25571.
8. Shiraishi, M.; Aramaki, Y.; Seto, M.; Imoto, H.; Nishikawa, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Nishimura, O.; Baba, M.; Fujino, M. *J. Med. Chem.* **2000**, *43*, 2049–2063.
9. Tarby, C. M.; Endo, N.; Moree, W.; Kataioka, K.; Ramirez-Weinhouse, M. M.; Imai, M.; Bradley, E.; Saunders, J. ACS meeting New Orleans, 1999, Poster Abstract 82.
10. Imai, M.; Shiota, T.; Kataoke, K.; Tarby, C.; Moree, W.; Tsutsumi, T.; Sudo, M.; Ramirez-Weinhouse, M.; Comer, D.; Sun, C. M.; Yamagami, S.; Tanaka, H.; Morita, T.; Hada, T.; Greene, J.; Barnum, D.; Saunders, J.; Myers, P.; Kato, Y.; Endo, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*(21), 5407–5411.
11. Moree, W.; Kataloka, K.; Ramirez-Weinhouse, M.; Shiota, T.; Imai, M.; Sudo, M.; Tsutsumi, T.; Endo, N.; Muroga, Y.; Hada, T.; Tanaka, H.; Morita, T.; Greene, J.; Barnum, D.; Saunders, J.; Kato, Y.; Myers, P.; Tarby, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*(21), 5413–5416.
12. Berkhout, T. A.; Blaney, F. E.; Bridges, A. M.; Cooper, D. G.; Forbes, I. T.; Gribble, A. D.; Groot, P. H. E.; Hardy, A.; Ife, R. J.; Rejbinder, K.; Moores, K. E.; Shillito, H.; Willetts, J.; Witherington, J. *J. Med. Chem.* **2003**, *46*, 4070–4086.
13. Yoshioka, M.; Kawakita, T.; Ohno, M. *Tetrahedron Lett.* **1989**, *30*(13), 1657–1660.
14. Human THP-1 cells (monocytic cell line, ATCC TIB-202) were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum (FCS), 1% L-glutamine, penicillin (50 U/mL) and streptomycin (50 g/mL) (all GIBCO BRL, Gent). After centrifugation, cells were loaded for 30 min with the Ca^{2+} sensitive fluorescent dye Fluo-3 AM (Molecular Probes, Leiden, Netherlands) (2 million cells/mL in RPMI medium containing 4 μM Fluo-3 AM, 20 mM HEPES, 0.1% bovine serum albumin (BSA) and 5 mM probenecid). Excess dye was removed by 3-fold washing with buffer (5 mM HEPES, 140 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl, 10 mM glucose, 2.5 mM probenecid, 1.25 mM $CaCl_2$, 0.1% BSA; all further incubations were done in this buffer). Cells were plated at a density of 150,000 cells/well in dark-wall 96-well plates (Costar, Cambridge, MA) and sedimented by centrifugation (1 min). The cells were pre-incubated for 20 min with test compound. Then, 10^{-7} M hMCP-1 (Bachem, Bubendorf, Switserland) was added. Changes in intracellular free Ca^{2+} concentration were measured using the Fluorescent Imaging Plate Reader (FLIPR, Molecular Devices, Munich, Germany). Fluorescence was recorded every second from 10 s before the addition of the MCP-1 untill 2 min after the addition (first minute: 60 records with 1 s intervals, second minute: 20 records with 3 s intervals). The maximal fluorescence obtained during this time frame was used for further calculations.
15. Mononuclear cells from human heparinized peripheral blood were isolated using Ficoll-Paque gradient centrifugation (Amersham Biosciences). Assays of chemotactic responsiveness were performed using disposable 96-well chemotaxis chambers (ChemoTx, Neuro Probe, Cabin John, MD) with 5 μm pore size polycarbonate (PVP-free) filter membranes. Mononuclear cells (5×10^6 cells/mL) were fluorescently labeled with 5 μg/mL Calcein-AM containing 0.05% F-127 (Molecular Probes, Eugene, OR) at 37 °C for 30 min. Labeled cells were washed twice and resuspended at 5×10^6 cells/mL in Hanks' Balanced Salt Solution (Gibco) supplemented with 0.2% bovine serum albumin. Subsequently, cells were pre-incubated for 10 min at room temperature with serial dilutions of the compounds in DMSO (final DMSO concentration of 0.2%). Bottom wells of the chemotaxis chamber were loaded with 28 μL medium containing 10 or 30 ng/mL recombinant hMCP-1 (R&D) or medium only. Pre-treated cells (100,000 cells) were added in triplicate to the topside of the filter (20 μL) and incubated at 37 °C in humidified air containing 5% CO_2. After 2 h incubation, the non-migrated cells were removed from the top of the filter by gently wiping the filter with a tissue. The migrated cells were measured using a fluorescent plate reader (excitation, 485 nm; emission, 538 nm). The chemotactic response is expressed as chemotactic index (CI), being the ratio of the means of migrated cells in the presence of MCP-1 and the means of migrated cells in the absence of chemokine.