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Discovery of a novel series of CXCR3 antagonists

Stefano Crosignani [a,*], Marc Missotten [a], Christophe Cleva [a], Ruggero Dondi [a], Yann Ratinaud [a], Yves Humbert [a], Ashis Baran Mandal [b], Agnès Bombrun [a], Christine Power [a], André Chollet [a], Amanda Proudfoot [a]

[a] Merck Serono SA, 9 Chem. des Mines, 1202 Geneva, Switzerland
[b] Syngene International Ltd, Biocon Park, Plot No. 2&3, Bommasandra IV Phase, Bommasandra–Jigani Link Road, Bangalore 560 099, India

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ABSTRACT

The discovery of a novel series of CXCR3 antagonists is described. Starting from an HTS positive, iterative optimization gave potent compounds (IC_{50} 15 nM in a chemotaxis assay). The strategy employed to improve the metabolic stability of these derivatives is described.

Chemokines (chemoattractant cytokines) are small (8–10 KDa) proteins responsible for directing leukocytes towards sites of inflammation.[1] They exert their signaling by interaction with chemokine receptors, which are members of the GPCR family. A number of chemokines/chemokine receptors have been associated with various autoimmune and inflammatory diseases.[1] In particular, the receptor CXCR3, which is mainly expressed on $CD4^+$ Th1 T cells, and its ligands CXCL9/Mig, CXCL10/IP-10, and CXCL11/I-TAC have been linked to diseases such as rheumatoid arthritis (RA),[2] multiple sclerosis (MS)[3] as well as allograft rejection,[4] and in general with pathologies that are associated with a major involvement of Th1 cells. For example, CXCR3 expression is increased in $CD4^+$ T cells of MS patients, and $CXCR3^+$ T cells accumulate in active lesions. Moreover the levels of CXCL10 are increased in the CSF of MS patients, and these levels correlate with the severity of the disease. Likewise, studies conducted on RA patients have showed that the majority of the infiltrating cells in synovial fluid express CXCR3 and the levels of IP-10 and Mig are elevated relative to normal individuals. Recently, the efficacy of the anti-IP10 antibody MDX1100 reported in a Phase 2 clinical trial for RA[5] reinforced the crucial role of the CXCL10-CXCR3 axis in this disease, and the potential for treatment that a small molecule CXCR3 antagonist could have.[6]

With the purpose to discover new CXCR3 antagonists, a high-throughput screening was run on 90,000 compounds, using a cAMP assay (CXCR3 being a Gαi-coupled receptor, its activation leads to a reduction in cAMP levels, as well as an increase in Ca^{2+} mobilization and actin polymerization, finally leading to cytoskeletal rearrangement and chemotaxis). One positive was found with submicromolar potency (**1**, Fig. 1). Characterization of compound **1** (Table 1) confirmed the potency in different assays, and the compound was found to be selective against other GPCRs. However, the in vitro profiling of the compound showed several severe liabilities: low solubility, low permeability in a Caco-2 assay, instability in acidic media and in particular high clearance in the presence of human and rat microsomes. Some of these liabilities could be linked to the presence of the acyl hydrazone moiety, which we decided to replace. A search was run for all structures present in our chemical collection or at commercial suppliers possessing various key features found in **1** but lacking the acylhydrazone moiety, and these compounds were screened. We were then delighted to find that the acylhydrazone moiety could be simply replaced with an amide group as in compound **2**, with a modest loss of potency.

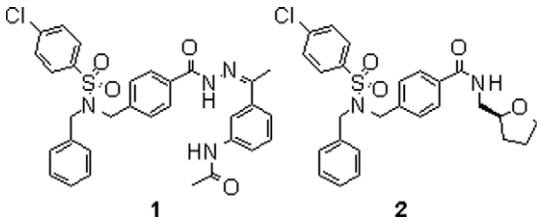

Figure 1. HTS positive **1** and Hit **2**.

* Corresponding author. Tel.: +41 224149618; fax: +41 227312179.
E-mail address: stefano.crosignani@merckserono.net (S. Crosignani).

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Table 1
Characterization of compound **1**

K_i (IP10/ITAC, nM)	348/211
GTPγS (IP10/ITAC, nM)	99/128
L1.2 Chemotaxis (IP10/ITAC, nM)	538/1810
Microsome Cl_{int} (human/rat) (μL/min/mg)	92/219
Caco-2 permeability (Papp, 10^{-6} cm/s)	0.1
Solubility (PBS, μM)[a]	11.9
Log *P*	4.8
Stability at pH 1	Unstable
MW	589

[a] Nephelometric kinetic solubility in PBS (1% DMSO).

Having found a more suitable starting point, we embarked in an optimization program. The nature of the scaffold made the chemistry strategy quite straightforward: depending on the point of the molecule that we wanted to diversify, one of the routes depicted in Scheme 1 could be applied: the sulfonamide **3** or **4** could be built first, followed by an alkylation in basic media, or the secondary amine **5** could be formed first, followed by reaction with a sulfonyl chloride, enabling the introduction of the diversity at a late stage of the synthesis. From carboxylic esters **6a** (X = COOMe), via the intermediate of the corresponding carboxylic acids **7**, amides and acylsulfonamides could be prepared, whereas starting from the corresponding nitriles **6b** (X = CN) a series of tetrazole compounds could be obtained.

To establish the SAR of this series, it was decided to use an assay that is physiologically relevant: a chemotaxis assay using CXCR3-overexpressing L1.2 cells and CXCL10 as chemoattractant was developed as previously described,[7] and was used throughout the project. The assay had a sufficient throughput to conduct the SAR, while being a direct measure of the ability of the compounds to inhibit the main pathophysiological result of CXCR3 activation, with the caveat that an overexpressing cell line was used instead of human T cells. The correlation of this assay with a more traditional binding assay was found to be very good (Fig. 2), giving at least an indirect proof that the effect of our compounds was on target. As negative control, a chemotaxis experiment on selected compounds was run using a different stimulus on appropriate cells (using CXCL13 on CXCR5-overexpressing L1.2 cells) and no effect on the resulting chemotaxis was observed (up to a concentration of 10 μM).

A quick optimization of amide **2** was performed on three diversity points (selected examples in Table 2). While some diversity was allowed for the amide N-substituent R^3, a preference for the more lipophilic groups and in particular for the thiophene methylene (**13**) and 1-phenyl-cycloprop-1-yl group groups (see **14** vs **11–12**, **26** vs **20–21**, **27** vs **23**) could be discovered.

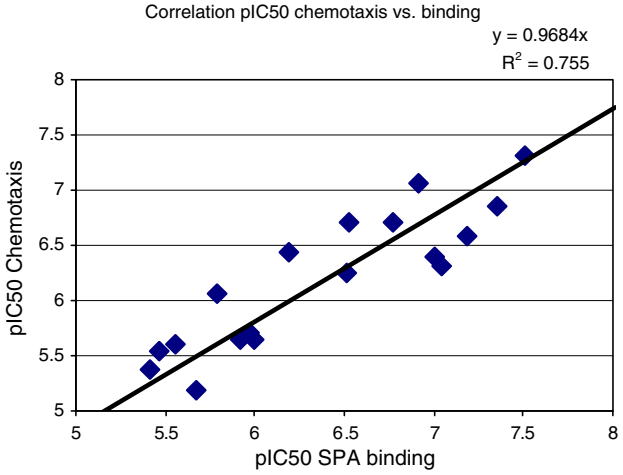
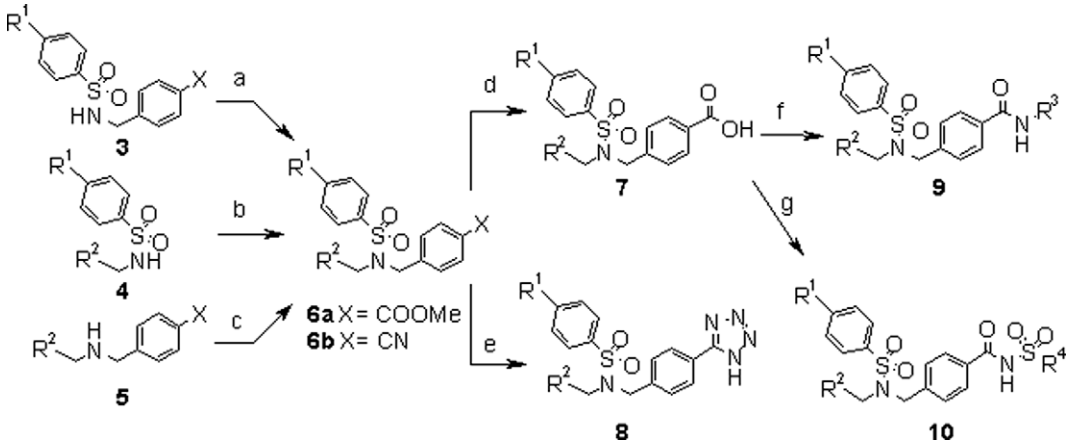


Figure 2. Correlation between activity in the L1.2 chemotaxis and binding assays.

On the other hand, substitution on the sulfonamide ring bearing R^1 proved to be limited: any kind of substitution in the *ortho* and *meta* positions of this aromatic group, as well as replacing the sulfonamide with an amide group, led to a severe decrease in potency (data not shown). The presence of the *para*-chlorine atom was important for potency, and good results could also be obtained substituting this chlorine atom with an alkoxy (**16**–**18**, **22** but not **19**) or a nitrile group (**23**, **27**). Finally, for the second benzyl substituent on the sulfonamide nitrogen R^2 the SAR was found again to be more tolerant, as long as an aromatic or heteroaromatic group was present with an unsubstituted methylene linker. In particular 2-pyridyl (see **20** vs **11**, **21** vs **12**) was the preferred substituents. On the other hand, aliphatic or cycloaliphatic groups were not tolerated (data not shown).

The most potent compound identified in this round of optimization was **27**, with an IC_{50} of 13 nM in the chemotaxis assay. However, it soon appeared that all these compounds shared a very serious liability, in that their stability in human and rat microsomes was very limited. The first strategy that was attempted to correct this issue was to block the most likely sites of metabolism. Some evidence from the microsome stability experiments allowed us to identify the methylene α to the amide bond as the principal site of metabolism. However, derivatives **14**, **26**, and **27,** in which



Scheme 1. Reagents and conditions: (a) or (b) R^2CH_2Cl, K_2CO_3, KI, DMF, 100 °C; (c) $ArSO_2Cl$, TEA, DCM; (d) NaOH, H_2O/THF; (e) Bu_2SnO, $TMSN_3$, toluene, 90 °C; (f) R^3NH_2, EDC, HOBt; (g) $R^4SO_2NH_2$, EDC, DMAP.

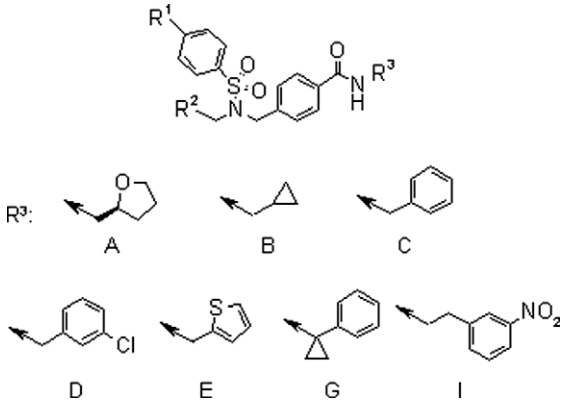


Figure 3. Compounds for Table 2.

Table 2
SAR of selected amides

Compounds	R^1	R^2	R^3	L1.2 Chemotaxis IC_{50}[a] (nM)	Microsome Cl_{int} (human/rat) (μL/min/mg)
2	Cl	Ph	**A**[b]	2275	490/1701
11	Cl	Ph	**B**	1985	—
12	Cl	Ph	**D**	1295	—
13	Cl	Ph	**E**	192	—
14	Cl	Ph	**G**	238	211/507
15	Cl	Ph	**I**	2450	—
16	OMe	Ph	**B**	697	—
17	OMe	Ph	**C**	347	—
18	OEt	Ph	**B**	673	—
19	OCF_3	Ph	**B**	5580	—
20	Cl	2-Py	**B**	200	265/538
21	Cl	2-Py	**D**	85	583/1191
22	OMe	3-Py	**B**	1152	—
23	CN	2-Py	**B**	360	—
24	Cl	2-F-Ph	**B**	510	—
25	Cl	3-Cl-Ph	**B**	940	—
26	Cl	2-Py	**G**	25	257/1256
27	CN	2-Py	**G**	13	435/474

[a] Average of two experiments. SD were generally within 50% of average.
[b] See Figure 3.

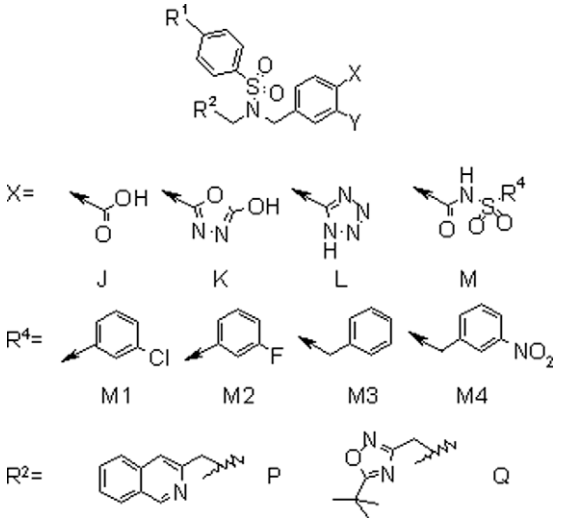


Figure 4. Compounds for Table 3.

Table 3
SAR of selected carboxylic acid, oxadiazolone, tetrazole and acylsulfonamide compounds

Compounds	R^1	R^2	X	Y	L1.2 Chemotaxis IC_{50}[a] (nM)	Microsome Cl_{int} (human/rat) (μL/min/mg)
28	Cl	Ph	**J**[b]	H	2940	<10/20
29	OMe	Ph	**J**	H	6440	—
30	Cl	2-Py	**J**	F	1132	—
31	Cl	2-F-Ph	**J**	F	513	ND/<10
32	CN	2-Py	**J**	F	1080	—
33	Cl	2-Py	**K**	H	695	—
34	Cl	2-Py	**L**	H	496	<10/25
35	Cl	2-F-Ph	**L**	H	470	—
36	Cl	3-Cl-Ph	**L**	H	1295	—
37	Cl	4-Cl-Ph	**L**	H	882	—
38	CN	2-Py	**L**	H	233	—
39	OEt	2-Py	**L**	H	540	—
40	Cl	4-(OMe)Ph	**L**	H	885	—
41	Cl	3-(OMe)Ph	**L**	H	1460	—
42	Cl	4-F-Ph	**L**	H	2560	—
43	Cl	P	**L**	H	911	—
44	Cl	Q	**L**	H	254	—
45	Cl	2-Py	**L**	F	440	—
46	OEt	2-Py	**L**	F	158	109/70
47	CN	2-F-Ph	**L**	F	192	ND/17[c]
48	Cl	2-F-Ph	**L**	F	157	<10/71
49	CN	2-F-Ph	**L**	H	236	ND/67
50	Cl	2-Py	**M1**	H	183	—
51	Cl	2-Py	**M2**	H	339	—
52	Cl	2-Py	**M3**	H	216	10/23
53	Cl	2-Py	**M4**	H	207	27/122

[a] Average of two experiments. SD were generally within 50% of average.
[b] See Figure 3.
[c] Cl_{int} in mouse microsomes.

this position was blocked, proved to still be unstable, with metabolism in this case happening on the left part of the molecule.

Another strategy was identified when we investigated compound **28** (Fig. 4 and Table 3). This intermediate in the synthesis of the corresponding amides was found to still be active on the receptor, albeit with reduced potency. More importantly, this compound was found to be stable in the presence of both human and rat microsomes. Since several metabolically labile positions still exist in this molecule, a possible explanation of this improvement is linked to the decrease in Log *D* of the molecule, which could reduce the affinity for the Cytochrome P450 isoforms responsible for the metabolism of the amide derivatives.

Several other carboxylic acid derivatives were tested, but only limited improvements in potency were obtained, the best compounds being those with a fluorine atom in *ortho* to the carboxylic acid (e.g., **31**).

In order to restore sufficient levels of potency on the receptor, a number of possible bioisosters of the carboxylic acid were also explored. Both tetrazole (**34**–**49**) and oxadiazolone (**33**) were found to be possible replacements, with a moderate gain in potency. SAR around these structures allowed the identification of several compounds with potency in the 100–200 nM range in the chemotaxis assay. The SAR was found to follow the same patterns recognized for the amide subseries, with a preference for a chlorine, alkoxy or nitrile substituent as R^1. For R^2, the 2-pyridyl was again found as a preferred substituent, but also the 2-fluorophenyl group was found to be equally potent. Although it was not possible to recover the low nanomolar IC_{50} obtained with the best amide compound, the tetrazoles were found to be generally more stable in microsomes than the corresponding amides, in keeping with the theory that the affinity of the compounds for the CYP isoform(s) is generally governed by their lipophilicity (with the possible exception of **46**, for which an alternative metabolic pathway involving O-dealkylation can be envisioned and which could be

due to a different CYP isoform). An additional advantage of the tetrazole compounds compared to the corresponding amides was a notable gain in aqueous solubility at pH 7.4: while all amides showed low solubility (<10 μM), all tested tetrazole compounds had solubilities greater than 100 μM.

Among the compounds profiled in PK experiments in mice compound **47** was identified as having the best profile, with a medium Clearance of 1.7 L/h/kg (ca. 30% of mouse liver blood flow) and a V_{ss} of 1.0 L/kg after iv dosing (0.2 mg/kg) and with a bioavailability of 83% and a half-life of 1 h after po dosing (10 mg/kg).

In a further effort to increase the potency while keeping good metabolic stability, we turned our attention to acylsulfonamides **50**–**53**, which would combine the presence of a negatively charged group at physiological pH (to increase metabolic stability and solubility) with the possibility to extend the molecule to occupy a similar region of space as occupied by the amide compounds (to try to restore low nanomolar potency). The compounds prepared showed potency on the receptor, but without a significant increase compared to the tetrazole compounds.

In summary, we have discovered a new series of CXCR3 antagonists. The first round of optimization yielded a potent compound (**27**, IC_{50} 13 nM), which was however unstable in microsomes. Lowering the Log *D* of the molecules by incorporating a negatively charged group (carboxylic acid, tetrazole or acylsulfonamide) led to active compounds with an improved stability versus oxidative metabolism. The best compound identified, **47**, while still having an insufficient potency and pharmacokinetic profile for using in a clinical setting, is a suitable lead compound for further optimization.

Supplementary data

Supplementary data (typical conditions for the synthesis of compounds, as well as experimental details on the in vitro assays. Full experimental conditions and characterization of all compounds can be found in patent WO2009124962) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.113.

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