CHEMMEDCHEM

DOI: 10.1002/cmdc.200700276

Discovery of Potent, Orally Bioavailable Small-Molecule Inhibitors of the Human CCR2 Receptor

Julien Doyon,* Erwin Coesemans, Staf Boeckx, Mieke Buntinx, Bart Hermans, Jean P. Van Wauwe, Ron A. H. J. Gilissen, Alex H. J. De Groot, David Corens, and Guy Van Lommen*[a]

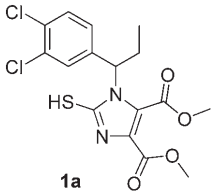
We recently reported the discovery of a series of 2-thioimidazoles as CCR2 antagonists. The most potent molecules of this series, the 4,5-diesters, were rapidly hydrolyzed to the inactive acids and were found to be metabolically unstable. Herein we describe the synthesis of a number of analogues with heterocyclic bioisosteric replacements of the ester group(s). Small 5-membered heterocyclic substituents at the 4-position gave highly potent CCR2 antagonists. Hydrolysis of the 5-ester is diminished, thus imparting these compounds with sufficient stability and systemic exposure after oral administration to warrant further study of the in vivo pharmacology of these functional CCR2 inhibitors.

Introduction

CCR2 is a G-protein-coupled chemokine receptor expressed mainly on monocytes, macrophages, dendritic cells, and some T lymphocytes. It is activated by high-affinity binding with the monocyte chemotactic proteins MCP-1–5. Among these proteins, only MCP-1 (or CCL2) interacts exclusively with CCR2 and is therefore recognized as the prime CCR2 ligand. In particular, the CCR2–MCP-1 interaction appears to be pivotal under inflammatory conditions. Indeed, CCR2- and MCP-1-deficient mice and CCR2 or MCP-1 antibody-treated rodents show decreased recruitment of monocytes and produce markedly attenuated inflammatory responses in animal models of multiple sclerosis, rheumatoid arthritis, atherosclerosis, diabetes, asthma, allograft rejection, and neuropathic pain. Moreover, clinical studies in humans have indicated an interdependence between MCP-1/CCR2 expression, monocyte accumulation, and disease symptoms in patients with multiple sclerosis, rheumatoid arthritis, pulmonary fibrosis, and crescentic nephritis.[1–4] Clearly, these observations confirm the role of CCR2 in the pathogenesis of several immune-based inflammatory diseases and identify this chemokine receptor as a potentially valuable therapeutic target.

A number of small-molecule antagonists of CCR2 have been reported recently,[4–11] and this has been summarized in a recent review by Feria and Díaz- González,[4] but only few data on their activity in in vivo models have been disclosed so far.[12] Although three reports have shown disappointing results with a CCR2 antagonist and anti-CCR2 and anti-MCP-1 antibodies in clinical trials in rheumatoid arthritis,[13–15] the development of small-molecule antagonists of the CCR2 receptor with improved bioavailability should provide the tools to better analyze the role of this target in in vivo inflammatory and other disease models, eventually leading to better treatment options.

We recently reported a new class of CCR2 receptor antagonists, with compound **1a** being the most potent example.[16] Although **1a** has an excellent pharmacodynamic profile in vitro, it was found to be rapidly metabolized in vivo through hydrolysis of the ester function in the blood plasma. We describe herein the synthesis of new equally potent analogues but with better plasma and hepatic metabolic stability.



1a

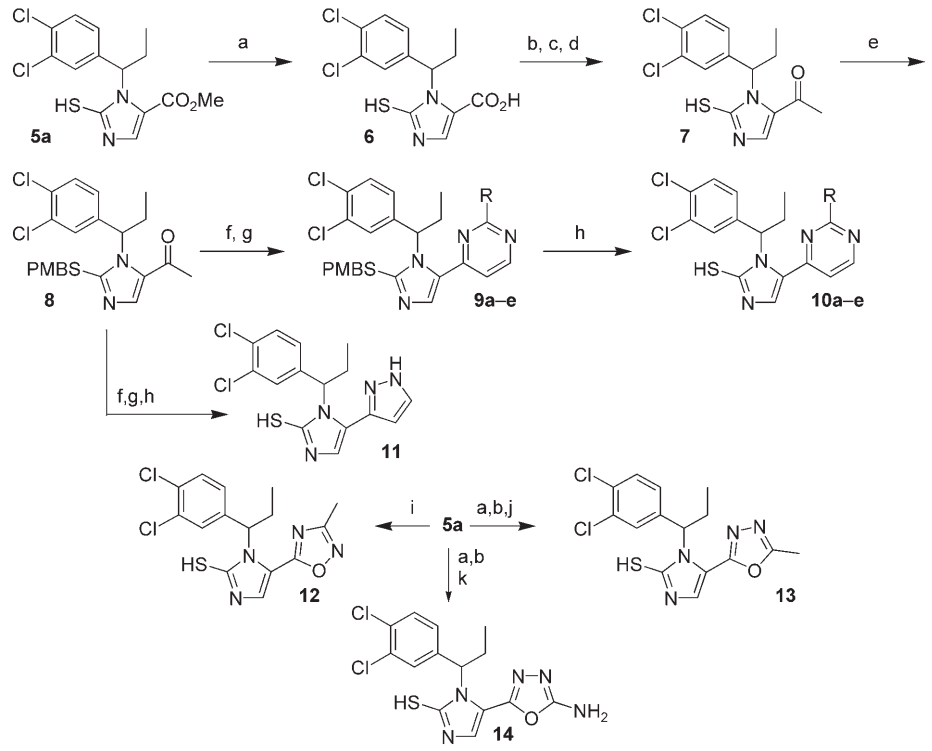
Results and Discussion

Chemistry

The SAR of the initial series clearly pointed to the importance of the 4,5-dicarboxylic substituents for activity. Although various substitution patterns led to compounds with either improved metabolic stability or high activity, the optimal combination was still lacking. To circumvent the sensitivity of the ester toward hydrolysis, we speculated that small heterocyclic systems might serve as good isosteric replacements for the esters, conferring improved plasma and hepatic metabolic stability, while preserving the key binding and electrostatic features.

Analogues without substituents at the 4-position were synthesized by further modification of the ester group of **5** (Scheme 1 and Figure 1). During the synthesis of some of the

[a] *Dr. J. Doyon, E. Coesemans, S. Boeckx, Dr. M. Buntinx, B. Hermans, Dr. J. P. Van Wauwe, Dr. R. A. H. J. Gilissen, Dr. A. H. J. De Groot, Dr. D. Corens, Dr. G. Van Lommen*
Johnson & Johnson Pharmaceutical Research and Development
Research and Early Development Europe
Turnhoutseweg 30, 2340 Beerse (Belgium)
Fax: (+32) 14-60-5344
E-mail: jdoyon@prdbe.jnj.com
gvlommen@prdbe.jnj.com



Scheme 1. Reagents and conditions: a) NaOH (1 N), CH_3OH, 80 °C; b) $SOCl_2$, CH_2Cl_2, reflux; c) *N*,*O*-dimethylhydroxylamine, THF, room temperature, overnight; d) MeMgBr, THF, 0 °C; e) NaH, THF, *p*-methoxybenzylbromide, room temperature; f) DMF–DMA, reflux, 12 h; g) Na, EtOH, guanidine or hydrazine, 100 °C (sealed tube), 20 h; h) TFA, 80 °C; i) acetamide oxime, NaOMe, CH_3OH (sealed tube), 100 °C, overnight; j) acetyl hydrazine, TEA, THF, overnight, then $POCl_3$, CH_3CN, 50 °C; k) semicarbazide·HCl, DIPEA, THF, room temperature, 18 h, then $POCl_3$, CH_3CN, 50 °C, 5 h. DMA = *N*,*N*-dimethylacetamide, PMB = *p*-methoxybenzyl.

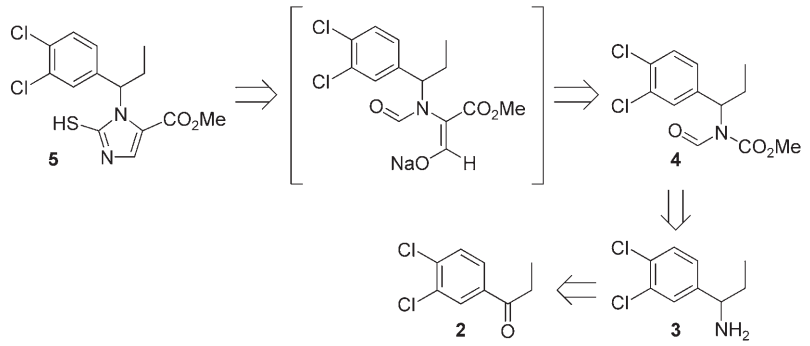
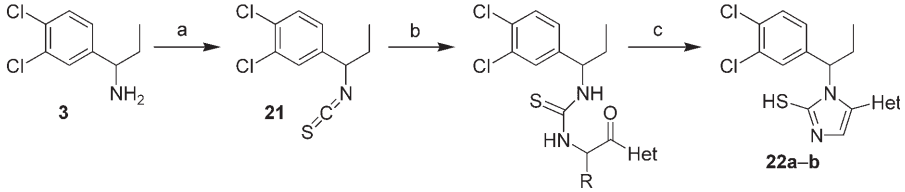


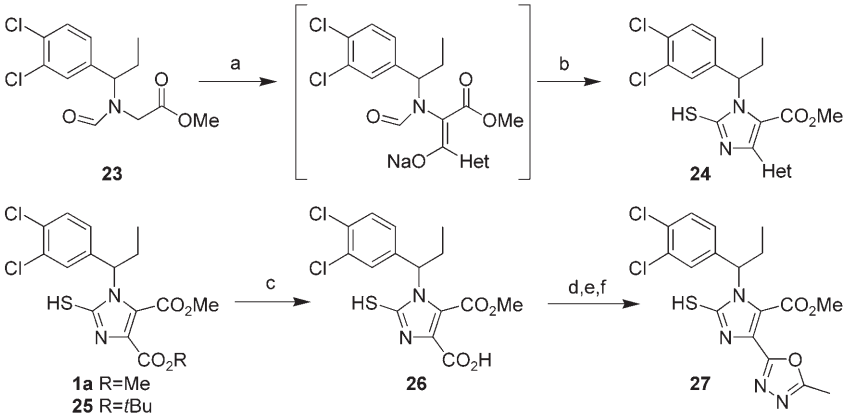
Figure 1. Retrosynthesis of compound **5**.

heterocycles that required electrophilic reagents, sulfur alkylation became a dominant side reaction, and a protection scheme had to be implemented (PMB/TFA). Scaffolds bearing the heteroaryl groups exemplified by **22a**–**b** were prepared as shown in Scheme 2.

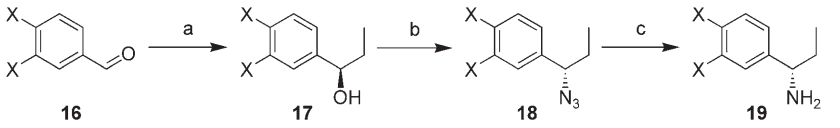
We then set out to examine analogues of the 4,5-disubstituted ester, which had shown the highest activity in the previous series (Scheme 3). Aryl or heteroaryl derivatives were prepared in low to moderate yield by condensation of the enolate of **23** with acid chlorides, leading to end-products **24a**–**x** (see Table 2 below). Critical in this scheme was the choice of the base, and the best yields were obtained with the use of lithium hexamethyldisilazide (LiHMDS). To derivatize the 4-position selectively, we initially chose to differentiate the two esters. When *tert*-butyloxalate was used in the condensation step, the 5-methyl-4-*tert*-butyl ester **25** was produced, in which the *tert*-butyl ester could be selectively hydrolyzed under acidic conditions to leave the 5-methyl ester untouched. Later we found that the 4-methyl ester **1a** could also be selectively hydrolyzed with 1 N NaOH at room temperature (Scheme 3). Quite notably the ester in the 5-position is very resistant to hydrolysis, and under harsher conditions decarboxylation becomes a competing side reaction. Further hydrolysis of the 5-ester group in these disubstituted compounds is again very difficult, with substantial decarboxylation and decomposition occurring, making further modification at that position problematic. Considering the high stability of this group and the difficulty in its derivatization, we felt that further functionalization via this route was not warranted. Enantiomers of **24e** and **24g** were also prepared, and their absolute configurations were established by the well-established enantioselective addition of diethylzinc to benzaldehyde. Alternatively, resolution of the amine via crystallization with a suitable chiral acid could be achieved (Scheme 4), and



Scheme 2. Reagents and conditions: a) thiophosgene, CH_2Cl_2; b) aminoketone, TEA, EtOH; c) HCl, dioxane, 75 °C, 4 h.



Scheme 3. Reagents and conditions: a) LiHMDS, THF, −78 °C, dimethyl oxalate; b) HCl (concd), CH_3OH, KSCN, reflux, 6–20 h; c) NaOH, CH_3OH, room temperature; d) $SOCl_2$, CH_2Cl_2; e) acetyl hydrazine; f) $POCl_3$, 60 °C.



Scheme 4. Reagents and conditions: a) Et_2Zn, toluene, *N*,*N*′-(1*S*,2*S*)-1,2-cyclohexanediylbis-[1,1,1]trifluoromethanesulfonamide, Ti(*i*PrO)$_4$, −78 °C; b) DPPA, DBU, toluene 0 °C→room temperature; c) H_2 Pd/C (5%), CH_3OH.

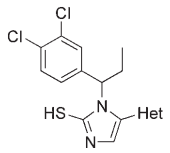
stereochemistry was confirmed by vibrational circular dichroism (VCD) analysis.[17]

Biological activity

The compounds were evaluated for their ability to inhibit MCP-1-induced calcium mobilization in human monocytic THP-1 cells (Tables 1 and 2). As hypothesized, small electron-rich heterocycles such as oxadiazoles **12**, **13**, and **14**, aminopyrimidines **10a** and **10e**, or pyrazole **11** all gave respectable activities in a range similar to the corresponding ester and amide[16] (pIC_{50}: 6.7 and 7.3, respectively). In contrast, more lipophilic substituents such as furyl or pyridyl (compounds **22a** and **22b**) were found to be only weakly active.

Similar trends were observed in the 4,5-disubstituted series for the replacement of the ester at the 4-position, and a broad variety of substituents is tolerated. Here again, the small heterocycles are preferred; isoxazole, triazole, pyridine, pyridazine, oxadiazole, or triazole are all highly active. The more polar or more lipophilic groups bring a sharp decrease in activity relative to the 4,5-diester **1a** (pIC_{50} ~ 8). Therefore, it appears that the above substituents can serve, at

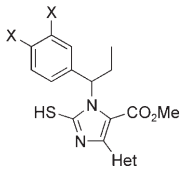
Table 1. pIC_{50} values determined in MCP-1-induced calcium mobilization assays for 4-Het-substituted thioimidazoles.



Compd	Het	pIC_{50}[a]
12	5-(3-methyl)-(1,2,4)-oxadiazolyl	6.7
13	2-(5-methyl)-(1,3,4)-oxadiazolyl	6.7
14	5-(2-amino)-(1,3,4)-oxadiazolyl	7.5
11	3-pyrazolyl	6.1
22a	2-furyl	6.1
22b	3-pyridyl	< 5
10a	4-(2-amino)pyrimidinyl	7.5
10b	4-(2-methyl)pyrimidinyl	6
10c	4-(2-(1-piperidinyl))pyrimidinyl	< 5
10d	4-(2-(4-morpholinyl))pyrimidinyl	5.5
10e	4-(2-methylamino)pyrimidinyl	7.4

[a] Results are the mean of 2–7 independent experiments.

Table 2. pIC_{50} values determined in MCP-1-induced calcium mobilization assays for 5-Het-substituted thioimidazoles.



Compd	X	Het	pIC_{50}[a]
24a	Cl	phenyl	6.7
24b	Cl	2-thiophenyl	7.2
27a	Cl	2-(5-methyl)-(1,3,4)-oxadiazolyl	7.5
27b	F	2-(5-methyl)-(1,3,4)-oxadiazolyl	6.9
24c	F	5-(4-methyl)-(1,2,3)-thiadiazolyl	7.5
24d	Cl	2-furyl	7.4
24e	Cl	5-isoxazolyl	7.9
24f	Cl	(*S*)-5-isoxazolyl	8.0
24g	F	5-isoxazolyl	7.9
24h	F	(*S*)-5-isoxazolyl	8.0
24i	F	(*R*)-5-isoxazolyl	5.9
24j	Cl	3-(5-methyl)isoxazolyl	7.9
24k	F	3-(5-methyl)isoxazolinyl	7.0
24l	F	4-(3,5-dimethyl)isoxazolyl	5.8
24m	Cl	2-pyridinyl	7.6
24n	Cl	3-pyridinyl	7.4
24o	Cl	4-pyridinyl	7.7
24p	Cl	2-pyridazinyl	7.6
24q	F	2-pyridazinyl	7.2
24r	Cl	5-(1,3-dimethyl)pyrazolyl	6.6
24s	Cl	3-(1,5-dimethyl)pyrazolyl	6.6
24t	Cl	2-(1-methyl)imidazolyl	7.5
24u	Cl	2-tetrahydrofuryl	6.6
24v	Cl	3-tetrahydrofuryl	5.9
24w	Cl	4-(1-methyl)piperidinyl	< 5
24x	Cl	3-(1-methyl)piperidinyl	< 5

[a] Results are the mean of 2–7 independent experiments.

least in the present context, as good isosteric replacements for a methyl ester function. As observed previously, the greatest activity is observed for the *S* enantiomers **24 f**.

A selection of these compounds was further evaluated in an assay measuring the chemotaxis of human peripheral blood mononuclear cells (PBMC) toward MCP-1 (Table 3). Most of the compounds also showed good activity in this assay, with **24 f** and **24 h** again being the most potent. The inhibitory effect on hCCR2/MCP-1 signaling was further confirmed in an MCP-1-induced calcium mobilization assay using human CCR2b-transfected Chinese hamster ovary (CHO) cells. In these cells, compound **24 h** inhibited calcium mobilization with a potency similar to that observed in the THP-1 cells ($pIC_{50} = 7.9 \pm 0.1$; data not shown). Finally, both **24 f** and **24 h** were found to be inactive against a panel of 51 receptors at a concentration of 1 μM (CEREP; data not shown).

Table 3. pIC_{50} values determined in dose–response assays measuring the inhibition of MCP-1-induced calcium mobilization in THP-1 cells and chemotaxis of human PBMC toward MCP-1.

Compd	pIC_{50} (Ca^{2+})[a]	pIC_{50} (chemotaxis)[a]
24 f	8.0	7.6
24 h	8.0	7.0
24 o	7.7	6.1
24 n	7.4	6.3
24 a	6.9	6.7

[a] Results are the mean of 2–7 independent experiments.

Although **1 a** was stable in liver microsomes, it was rapidly hydrolyzed in human and mouse plasma. On the other hand, **24 f** and **24 h** were both stable in liver microsomes as well as in human and mouse plasma. In addition, metabolite identification performed on the incubated samples did not indicate loss of the ester group and did not reveal the acid metabolites either. We emphasize here the agreement between chemical and biological resistance to hydrolysis of the 5-ester groups in these molecules.

Key pharmacokinetic (PK) data of **24 f** and **24 h** in rat are shown in Table 4. Both compounds display a reasonable bioavailability after oral administration with some variability for **24 f**; they are rapidly absorbed, with a T_{max} of 30 min and have a moderate clearance. The improvement in metabolic stability for both compounds is reflected in a half-life of 4–7 h after oral administration. Given the close resemblance between these two compounds, their difference in the volume of distribution is noteworthy.

Table 4. Plasma kinetics after single i.v. (2.5 mg kg^{-1}) and oral (10 mg kg^{-1}) administration of CCR2 antagonists in male SPF Sprague–Dawley rats.

PK Parameter	**24 f**	**24 h**
C_{max} [ng mL^{-1}] p.o.	968	597
T_{max} [h] p.o.	0.5	0.5
$t_{1/2\,(4-24)}$ [h] p.o.	7.3	4.1
$AUC_{0-24\,h}$ [ng h mL^{-1}] p.o.	1975	2164
Clearance [L h^{-1} kg^{-1}] i.v.	1.90	1.87
V_d [L kg^{-1}] i.v.	1.59	9.05
$AUC_{0-24\,h}$ [ng h mL^{-1}] i.v.	1130	1422
Oral bioavailability [Fabs, %]	16–42	41

[a] Data are given as the mean of three animals.

Conclusions

Although **1 a** is a potent antagonist of the human CCR2 receptor, its rapid hydrolysis in plasma precludes its use in in vivo studies. Substitution of the 4-ester by various heterocyclic isosteres gives compounds with potent CCR2 antagonist properties and with high metabolic stability in both plasma and liver, as exemplified with **24 f** and **24 h**. As such, compounds **24 f** and **24 h** appear to have sufficient oral bioavailability to warrant evaluation of the functional role of CCR2 antagonists in vivo. Indeed, further pharmacological studies on these human CCR2 antagonists will be reported in the near future.

Experimental Section

Analytical procedures

NMR: ^1H and ^{13}C NMR spectra were recorded on Bruker Avance-II 600, DPX 360, DPX 400 and AMX 400 spectrometers. $CDCl_3$ was used as solvent, unless mentioned otherwise. The chemical shifts are expressed in ppm relative to tetramethylsilane. Carbon signals are singlets, unless mentioned otherwise.

High-resolution mass spectrometry (HRMS): HRMS was measured on a QToF mass spectrometer (Waters, Milford, USA). Data were acquired from 50 to 800 Da using a desolvation temperature of 380 °C and a source temperature of 120 °C. Cone voltage was 30 V and collision energy was 5 eV. Data peak centroids were referenced during acquisition using an external reference (LockSpray) of leucine encephalin (2 μg mL^{-1}) infused at 3 μL min^{-1} to generate $[M+H]^+$ *m/z* 556.2771.

Optical rotation (OR): OR data were obtained on a PerkinElmer polarimeter, and melting points (mp) were measured in open capillaries (Büchi).

Synthetic procedures

Synthesis of 5: A solution of sodium carbonate (54.8 g) in 250 mL H_2O was added to a stirred mixture of 1-(3,4-dichlorophenyl)propan-1-one (70 g) in 150 mL EtOH, and $NH_2OH{\cdot}HCl$ (29.4 g) was added portion-wise while stirring vigorously. The reaction mixture was heated at reflux for 8 h, after which 5.7 g extra $NH_2OH{\cdot}HCl$ was added; the reaction mixture was held at reflux for a further 24 h and then stirred for 2 days at room temperature. The solids were filtered off, washed with EtOH/H_2O (1:1) and dried (vacuum, stream of air) at 56 °C. Yield: 71.8 g 3,4-dichlorophenylpropiophenoneoxime.

3,4-dicholorophenylpropiophenone oxime (67 g) in 500 mL EtOH saturated with NH_3 was hydrogenated at 14 °C with Raney nickel (cat.) in the presence of a solution of thiophene (6 mL, 1%). After uptake of 2 equiv H_2, the catalyst was filtered off, and the filtrate was evaporated, then co-evaporated twice with toluene. The residue was stirred in boiling 2-propanol (250 mL), and the mixture

was filtered off hot. The filtrate was allowed to reach room temperature, and HCl/2-propanol (6 N, 150 mL) was added slowly while stirring vigorously. The solvent was evaporated and the residue was stirred in *N*,*N*-diisopropylethylamine (DIPEA), then filtered off, washed and dried in vacuo at 60 °C; yield: 53 g (73.4%). A part of this fraction was converted into its free base: the salt was stirred in CH_2Cl_2 (200 mL), a solution of K_2CO_3 (15%, aq) was added, the resulting mixture was then stirred for 1 h, and a solution of NaOH (50%) was added to increase the pH. The organic layer was separated, washed with H_2O, dried ($MgSO_4$), filtered off, and the solvent was evaporated to yield **3** quantitatively.

A solution of 1-(3,4-dichlorophenyl)-1-aminopropane (18 g) and methyl chloroformate (12.3 mL) in *N*,*N*-dimethylformamide (DMF, 150 mL) was stirred at room temperature under N_2, and triethylamine (TEA, 31 mL) was slowly added. The reaction mixture was then stirred for 20 h at room temperature, and 3.3 mL extra methyl chloroformate was added. The resulting mixture was stirred for another 24 h, and the solids were then filtered off and washed with DMF. Et_2O (800 mL) was added, and the mixture was washed three times with H_2O (500 mL). The organic layer was separated, dried over $MgSO_4$, and the solvent was evaporated, then co-evaporated with toluene. The residual oil (23.4 g) was purified over silica gel (eluent: CH_2Cl_2/CH_3OH 99:1). The product fractions were collected, and the solvent was evaporated; final co-evaporation with toluene yielded 20.6 g 1-(3,4-dichlorophenyl)-1-(methylglycinate)propane.

A solution of formic acid (7.5 mL) and 1-(3,4-dichlorophenyl)-1-(methylglycinate)propane (20.6 g) in xylene (225 mL) was stirred and held at reflux for 4 h, and then the reaction mixture was cooled to room temperature. The mixture was washed with H_2O (2×200 mL), $NaHCO_3$ (200 mL, aq, saturated), and brine (200 mL). The separated organic layer was then dried over $MgSO_4$ and filtered off. Finally, the solvent was evaporated. Yield: 21.3 g **4** (93.9%).

NaOMe (3.8 g) was added to a stirring solution of **4** (21 g) and methyl formate (11.4 g) in 100 mL THF under N_2, and the reaction mixture was stirred at room temperature for 40 h. The solvent was evaporated, and the crude residue was stirred in H_2O (90 mL). The aqueous layer was washed with Et_2O (2×50 mL), and CH_3OH (60 mL) was added. The solution was heated at 45 °C for 24 h, then KSCN (10.7 g) was added. The mixture was stirred at room temperature for 40 h and at 80 °C for 5 h. The reaction mixture was cooled to room temperature, and the solids were filtered off, washed with H_2O/CH_3OH (2:1), then dried at 60 °C (in vacuo). Yield: 19.35 g. The entire fraction was recrystallized from CH_3CN (200 mL), filtered off, washed with CH_3CN, and dried at 50 °C (in vacuo). Yield: 17.2 g **5** (75.5%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 13.16 (1H, brs), 7.94 (1H, s), 7.56 (1H, d, J = 8.4 Hz), 7.43 (1H, s), 7.16 (1H, d, J = 8.4 Hz), 6.43–6.54 (1H, m), 3.64 (3H, s), 2.18–2.76 (2H, m), 0.78 ppm (3H, t, J = 7.3 Hz); ^{13}C NMR (101 MHz, [D$_6$]DMSO): δ = 167.11, 157.96, 141.13, 130.60, 130.06, 129.29, 128.32, 126.80, 126.00, 119.58, 58.58, 51.70, 23.09, 10.66 ppm; HRMS $[M+H]^+$ *m/z* calcd 345.0231, obsd 345.0237.

Synthesis of 12: **5** (250 mg) and *N*-hydroxyacetamidine (200 mg) in CH_3OH (3 mL) and NaOMe (1 mL, 30%) were allowed to react at 100 °C in a sealed tube for 18 h, and then the reaction mixture was quenched with NH_4Cl (aq). The solvent was evaporated, and the residue was extracted with CH_2Cl_2. The organic layer was separated, filtered through a drying cartridge, and the solvent was evaporated. The residue was purified by HPLC; the product fractions were collected, and the solvent was evaporated. Yield: 0.110 g **12**. ^{1}H NMR (360 MHz): δ = 11.29 (1H, brs), 7.65 (1H, s), 7.54 (1H, d, J = 2.1 Hz), 7.35 (1H, d, J = 8.4 Hz), 7.23 (1H, dd, J = 8.4, 2.1 Hz), 6.64 (1H, dd, J = 10.5, 5.6 Hz), 2.92 (1H, brs), 2.41–2.53 (1H, m), 2.39 (3H, s), 0.98 (3H, t, J = 7.3 Hz); mp: 227.5 °C; HRMS $[M+H]^+$ *m/z* calcd 369.0344, obsd 369.0342.

Synthesis 13: **5** (3 g) was stirred in CH_3OH (40 mL), and NaOH (40 mL, 1 N) was added. After 18 h at 80 °C, the solvent was evaporated. H_2O was added, and the mixture was acidified with HCl, the precipitate was filtered off, washed with H_2O, and dried to give 2.3 g **6**. ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 13.04 (1H, brs), 12.94 (1H, brs), 7.84 (1H, d, J = 2.8 Hz), 7.56 (1H, d, J = 8.4 Hz), 7.41 (1H, brs), 7.13 (1H, d, J = 8.5 Hz), 6.48 (1H, dd, J = 10.3, 5.7 Hz), 2.32–2.68 (2H, m), 0.78 ppm (3H, t, J = 7.3 Hz).

Compound **6** (0.33 g) was treated with thionyl chloride (10 mL) and held at reflux for 2 h. The thionyl chloride was evaporated, toluene (25 mL) was added twice and evaporated to give 0.35 g of the corresponding acid chloride. Acetyl hydrazine (150 mg) and TEA (0.28 mL) were stirred in 15 mL THF, and the acid chloride obtained above in 5 mL THF was added dropwise. The mixture was stirred overnight, the solvent was evaporated, and the residue was treated with H_2O and extracted with CH_2Cl_2. The compound precipitated from the biphasic mixture and was filtered off to give 160 mg of the corresponding acetyl hydrazide.

A mixture of the acetyl hydrazide (76 mg) obtained above in CH_3CN (2 mL) was stirred in a closed vessel, and $POCl_3$ (23 mL) was added. The reaction mixture was then stirred at 90 °C for 1.5 h, H_2O (5 mL) was added, and the mixture was extracted with CH_2Cl_2/CH_3OH (90:10). The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was purified by silica gel chromatography (eluent: CH_2Cl_2/CH_3OH 95:5), and the product fractions were collected, the solvent was evaporated, and the residue was dried in vacuo. Yield: 35 mg **13**. ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 13.17 (1H, brs), 7.90 (1H, d, J = 2.1 Hz), 7.56 (1H, d, J = 8.4 Hz), 7.48 (1H, s), 7.20 (1H, d, J = 8.4 Hz), 6.51 (1H, dd, J = 10.8, 5.4 Hz), 2.67 (1H, brs), 2.46 (3H, s), 2.33–2.45 (1H, m), 0.80 ppm (3H, t, J = 7.3 Hz); mp: 227.5 °C; HRMS $[M+H]^+$ *m/z* calcd 369.0344, obsd 369.0313.

Synthesis of 14: The acid chloride obtained with the method above (5.2 mmol) was dissolved in THF under N_2 and treated successively with DIPEA (2.8 mL) and semicarbazide·HCl (0.87 g). The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was taken up in K_2CO_3 (aq) and washed with CH_2Cl_2/CH_3OH 95:5. The aqueous layer was acidified with HCl (concd), and the precipitate formed was collected, washed with H_2O, and dried to give 0.42 g product. This product (0.35 g) in CH_3CN (15 mL) was treated with $POCl_3$ (0.1 mL) and heated at 90 °C for 2 h. After cooling, the reaction mixture was poured into $NaHCO_3$ (aq) and extracted with CH_2Cl_2/CH_3OH 98:2. The organic extract was dried, concentrated, and the residue was purified by reversed-phase (RP) HPLC to give 69 mg product **14** as a solid. ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 12.91 (brs), 7.57 (s), 7.55 (d, J = 8.5 Hz), 7.44 (brs), 7.29 (brs), 7.16 (d, J = 8.5 Hz), 6.43 (dd, J = 10.7, 5.5 Hz), 2.72 (brs), 2.31–2.43 (m), 0.82 ppm (t, J = 7.2 Hz).

Synthesis of 11: A mixture of *N*-methoxymethylamine (500 mg) and TEA (500 mg) in 10 mL THF was stirred at room temperature, and a mixture of 500 mg of the acid chloride of **5** in 5 mL THF was added dropwise over 15 min. The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was stirred in H_2O, and the mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was dissolved in acetone (15 mL, p.a.) and a stream of SO_2 was bubbled through the

solution for 20 min. The solvent was evaporated, and the residue was filtered over silica gel (eluent: CH_2Cl_2/CH_3OH 95:5). The product fractions were collected, and the solvent was evaporated. Yield: 310 mg of the corresponding *N*-methoxymethylamide. ^{1}H NMR (360 MHz, [D_6]DMSO): $\delta = 12.89$ (1H, br s), 7.53–7.59 (2H, m), 7.41 (1H, d, $J = 2.1$ Hz), 7.19 (1H, dd, $J = 8.4$, 2.1 Hz), 6.23 (1H, dd, $J = 10.0$, 5.9 Hz), 3.51 (3H, s), 3.08 (3H, s), 2.47 (1H, br s), 2.26–2.39 (1H, m), 0.81 ppm (3H, t, $J = 7.2$ Hz).

A mixture of the compound prepared above (300 mg) in 10 mL THF was stirred at 0–5 °C, and methyl magnesium chloride (30% in THF, 3 mL) was added dropwise followed by an additional 6 mL after 1 h. The reaction mixture was stirred again for 1 h and quenched with HCl (1 N, 10 mL). H_2O was added, and the mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was dissolved in acetone, and a stream of SO_2 was passed through the solution for 20 min. The solvent was evaporated, and the residue was purified by flash chromatography on silica gel (eluent: EtOAc). The product fractions were collected and dissolved in CH_2Cl_2/CH_3OH (90:10). The mixture was then filtered, and the solvent was evaporated. Yield: 60 mg **7**. ^{1}H NMR (360 MHz, [D_6]DMSO): $\delta = 13.22$ (1H, br s), 8.34 (1H, s), 7.53 (1H, d, $J = 8.4$ Hz), 7.37 (1H, br s), 7.10 (1H, d, $J = 8.5$ Hz), 6.40–6.51 (1H, m), 2.37 (2H, br s), 2.27 (3H, s), 0.72 ppm (3H, t, $J = 7.3$ Hz).

A mixture of **7** (1.72 g) in THF (40 mL) was stirred at room temperature, and NaH (60%, 220 mg) was added portion-wise over 10 min. The mixture was stirred for 30 min, and a solution of *p*-methoxybenzyl chloride (860 mg) in THF (10 mL) was added immediately. The reaction mixture was stirred at room temperature for 6 days, and the solvent was evaporated. The residue was stirred in H_2O and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. Yield: 2.2 g **8**. A mixture of **8** (320 mg) and dimethylformamide dimethyl acetal (10 mL) was heated at 100 °C for 3 h. The solvent was evaporated, and the residue was filtered over silica gel (eluent: CH_2Cl_2/CH_3OH 98:2). The product fractions were collected, and the solvent was evaporated at 50 °C under a stream of N_2. Yield: 0.580 g. A mixture of the compound obtained above (580 mg) and hydrazine (350 mg) in EtOH (20 mL) was stirred and held at reflux (80 °C) for 3 h. The solvent was evaporated, and the residue was purified over silica gel (eluent CH_2Cl_2/EtOH 98:2). The product fractions were collected and dried under N_2 atmosphere to give 240 mg protected **11**.

A mixture of this compound (120 mg) in trifluoroacetic acid (TFA, 2 mL) was stirred in a closed vessel at 80 °C for 20 h and then the solvent was evaporated at 80 °C under a stream of N_2. H_2O (1 mL) and CH_2Cl_2 (5 mL) were added to the residue, and the mixture was treated with K_2CO_3; the layers were then separated by Isolute SPE. The organic layer was evaporated, and the residue was purified by flash chromatography over silica gel (eluent: EtOAc/hexanes 75:25). The product fractions were collected, the residue was stirred overnight in hexanes, and the solvent was evaporated again. After solidification the desired product was dried. Yield: 36 mg **11**. ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta = 12.76$ (1H, br s), 12.03 (1H, br s), 7.63 (1H, br s), 7.45 (1H, d, $J = 8.4$ Hz), 7.39 (1H, br s), 7.19 (1H, d, $J = 8.2$ Hz), 7.02 (1H, s), 6.34 (1H, dd, $J = 9.3$, 5.3 Hz), 6.11 (1H, br s), 2.59 (1H, br s), 2.16–2.29 (1H, m), 0.81 ppm (3H, t, $J = 7.3$ Hz); HRMS $[M+H]^+$ *m/z* calcd 353.0394, obsd 353.0404.

Synthesis of 22a: DIPEA (26.4 mL) was added to a stirring mixture of 1-(3,4,-dichlorophenyl)-1-aminopropane (10 g) in CH_2Cl_2 (100 mL) under N_2. After 15 min stirring, the reaction mixture was put on an ice bath, and a solution of thiophosgene (3.5 mL) in CH_2Cl_2 (15 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 30 min, then at room temperature for 18 h. The mixture was washed twice with H_2O, once with HCl (1 N) and again with H_2O. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated, then co-evaporated with toluene. The residue was purified by column chromatography over silica gel (eluent: CH_2Cl_2/hexanes 15:85). The product fractions were collected, and the solvent was evaporated. Yield: 7.4 g **21**.

2-Amino-2-furoylacetamide (34 mg), H_2O (0.5 mL), and K_2CO_3 (21 mg) were added to a solution of **21** (38 mg) in 2.5 mL dioxane, and the reaction mixture was stirred vigorously for 20 h. HCl (0.5 mL, concd) was added, and the resulting mixture was stirred for 4 h at 75 °C and then allowed to reach room temperature. H_2O was added, and the mixture was extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was purified by RP HPLC (R-STAN). The desired product fractions were collected, and the organic volatiles were evaporated. The product was extracted with CH_2Cl_2, and the separated organic layer was evaporated. Yield: 7.6 mg **22a**. ^{1}H NMR (360 MHz): $\delta = 11.42$ (1H, br s), 7.39 (1H, d, $J = 1.8$ Hz), 7.32 (1H, d, $J = 8.4$ Hz), 7.25 (1H, d, $J = 2.2$ Hz), 7.06 (1H, dd, $J = 8.4$, 2.2 Hz), 6.86 (1H, s), 6.34 (1H, dd, $J = 3.3$, 1.8 Hz), 6.20–6.32 (1H, m), 6.06 (1H, d, $J = 3.3$ Hz), 2.02–2.29 (2H, m), 0.97 ppm (3H, t, $J = 7.3$ Hz).

Compounds **22b** was synthesized by the same procedure: ^{1}H NMR (400 MHz): $\delta = 10.99$ (1H, br s), 8.62 (1H, dd, $J = 4.9$, 1.7 Hz), 8.24 (1H, d, $J = 2.3$ Hz), 7.35 (1H, d, $J = 8.4$ Hz), 7.12–7.18 (2H, m), 7.01 (1H, dd, $J = 8.4$, 2.2 Hz), 6.96 (1H, d, $J = 8.0$ Hz), 6.70 (1H, s), 6.26–6.36 (1H, m), 2.02–2.16 (1H, m), 1.82–1.93 (1H, m), 0.95 ppm (3H, t, $J = 7.3$ Hz); ^{13}C NMR (101 MHz): $\delta = 164.20$, 150.65, 150.56, 139.45, 138.03, 132.68, 132.05, 130.43, 129.18, 127.97, 126.84, 124.56, 122.62, 114.30, 59.26, 24.39, 10.61 ppm; HRMS $[M+H]^+$ *m/z* calcd 364.0442, obsd 364.0450.

Synthesis of 10a: A mixture of Na (130 mg) in 4 mL EtOH was stirred under N_2 at room temperature until complete dissolution, guanidine (720 mg) was added, and the mixture was stirred for 30 min. A mixture of **8** (277 mg) in 1 mL EtOH was added. The reaction mixture was stirred in a closed vessel at 100 °C for 20 h, and the solvent was evaporated. The resulting residue was purified by column chromatography on silica gel (eluent: CH_2Cl_2/CH_3OH 98:2). The product fractions were collected, and the solvent was evaporated. The residue was dissolved in 2-propanol and converted into the hydrochloric acid salt (1:1) with HCl/2-propanol. The crystallized salt was filtered off, washed with diisopropylether (DIPE), and dried. Yield: 210 mg **9a**.

A mixture of **9b** (210 mg) in TFA (3.5 mL) was stirred at 80 °C in a closed vessel for 4 h, and then the solvent was evaporated at 80 °C under a stream of N_2. The residue was stirred in CH_2Cl_2 and treated with H_2O and K_2CO_3. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was stirred in EtOAc (2 mL), and after filtration the desired product was dried. Yield: 20 mg **10a**. ^{1}H NMR (360 MHz, [D_6]DMSO): $\delta =$ 12.79 (1H, br s), 8.10 (1H, d, $J = 5.2$ Hz), 7.79 (1H, d, $J = 2.4$ Hz), 7.47 (1H, br s), 7.49 (1H, d, $J = 8.5$ Hz), 7.18 (1H, d, $J = 8.4$ Hz), 6.75 (1H, br s), 6.70 (1H, br s), 6.49 (2H, br s), 2.89 (1H, br s), 2.41 (1H, br s), 0.83 ppm (3H, t, $J = 7.3$ Hz); mp: 85.5 °C; HRMS $[M+H]^+$ *m/z* calcd 380.0503, obsd 380.0480.

Compounds **10b**, **10c**, **10d**, and **10e** were synthesized by a similar procedure.

10b: ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 13.02 (1H, brs), 8.56 (1H, d, J = 5.4 Hz), 8.03 (1H, s), 7.51 (1H, d, J = 5.4 Hz), 7.52 (1H, d, J = 8.5 Hz), 7.47 (1H, brs), 7.15 (1H, d, J = 8.5 Hz), 6.63 (1H, dd, J = 10.0, 5.9 Hz), 2.57 (1H, brs), 2.40–2.49 (1H, m), 2.35 (3H, s), 0.77 ppm (3H, t, J = 7.3 Hz).

10c: ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 2.87 (1H, brs), 8.28 (1H, d, J = 5.1 Hz), 7.82 (1H, s), 7.56 (1H, d, J = 8.4 Hz), 7.40 (1H, brs), 7.13 (1H, d, J = 8.5 Hz), 6.81 (1H, d, J = 5.1 Hz), 6.48–6.60 (1H, m), 3.24 (4H, brs), 2.30–2.45 (1H, m), 1.44–1.58 (2H, m), 1.26 (4H, brs), 0.69 ppm (3H, t, J = 7.2 Hz); mp: 184.3 °C; HRMS [M+H]$^+$ m/z calcd 448.1129, obsd 448.1113.

10d: ^{1}H NMR (360 MHz, [D$_6$]DMSO): δ = 12.92 (1H, brs), 8.33 (1H, d, J = 5.1 Hz), 7.86 (1H, s), 7.57 (1H, d, J = 8.4 Hz), 7.41 (1H, s), 7.12 (1H, d, J = 8.5 Hz), 6.91 (1H, d, J = 5.2 Hz), 6.45–6.55 (1H, m), 3.37–3.49 (4H, m), 3.17 (4H, brs), 2.33–2.46 (2H, m), 0.68 ppm (3H, t, J = 7.2 Hz); mp: 151 °C; HRMS [M+H]$^+$ m/z calcd 450.0922, obsd 450.0919.

10e: ^{1}H NMR (400 MHz, [D$_6$]DMSO, 100 °C): δ = 12.29 (brs), 8.16 (d, J = 5.1 Hz), 7.52 (s), 7.41–7.47 (m), 7.21 (d, J = 8.4 Hz), 6.70 (dd, J = 10.2, 5.7 Hz), 6.66 (d, J = 5.1 Hz), 6.56 (brs), 2.78–2.90 (m), 2.64 (d, J = 4.8 Hz), 2.31–2.44 (m), 0.84 ppm (t, J = 7.3 Hz); HRMS [M+H]$^+$ m/z calcd 394.0660, obsd 394.0660.

Synthesis of 24h: A solution of (1*S*,2*S*)-1,2-bis(trifluoromethanesulfonamido)cyclohexane (91 mg) and titanium tetraisopropoxide (122.4 mL) in 500 mL toluene was degassed under Ar and then stirred for 20 min at 40 °C. This mixture was cooled to −78 °C, and 42.5 mL diethylzinc (pure) was added dropwise over 15 min. The resulting mixture was stirred for 15 min at −78 °C, and then a solution of 3,4-difluorobenzaldehyde (**16**, X = F) in toluene was added dropwise over 20 min. The reaction mixture was stirred for a further 30 min at −78 °C, and was then allowed to slowly reach 0 °C. The mixture was quenched with 1 N HCl and extracted with CH_2Cl_2. Both organic and aqueous layers were filtered over dicalite, and the organic layer in the obtained filtrate was separated, then washed with H_2O, dried ($MgSO_4$), and filtered off. Finally, the solvent was evaporated. Yield: 57.7 g (*R*)-1-(3,4-difluorophenyl)-1-propanol (**17**). ^{1}H NMR (360 MHz): δ = 7.17 (1H, ddd, J = 11.3, 7.7, 2.1 Hz), 7.12 (1H, dt, J = 10.1, 8.1 Hz), 7.00–7.06 (1H, m), 4.57 (1H, t, J = 6.5 Hz), 1.99 (1H, s), 1.62–1.85 (2H, m), 0.90 ppm (3H, t, J = 7.4 Hz); OR +30.87° (589 nm, $CHCl_3$, 20 °C).

The alcohol (57 g) and diphenylphosphoryl azide (DPPA, 103 g) in 500 mL toluene was stirred under N_2 at 0 °C. 1,8-Diazabicyclo-[5.4.0]undec-7-ene (DBU, 60.3 mL) was then added dropwise, and the reaction mixture was stirred for 1 h at 0 °C. The mixture was stirred for 1 h at 45 °C, overnight at room temperature, and then poured out into H_2O (500 mL) and extracted with diethyl ether. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. The residue was filtered over silica gel (eluent: CH_2Cl_2 100%). The product fractions were collected, and then the solvent was evaporated and co-evaporated with toluene. Yield: 57.2 g (*S*)-azide **18**. ^{1}H NMR (360 MHz): δ = 7.09–7.21 (2H, m), 6.98–7.05 (1H, m), 4.32 (1H, t, J = 7.1 Hz), 1.67–1.91 (2H, m), 0.92 ppm (3H, t, J = 7.4 Hz); OR −109° (589 nm, $CHCl_3$, 20 °C).

A mixture of azide (57.5 g) in CH_3OH (600 mL) was hydrogenated with Pd/C (2 g, 10%) as a catalyst. After disappearance of the azide, the catalyst was filtered off, and the filtrate was evaporated. The residue was dissolved in CH_2Cl_2 (300 mL) and then 1 N HCl was slowly added while the solution was stirred vigorously on an ice bath. After this extraction, the aqueous layer was adjusted to pH > 10 with NaOH (50%), and the resulting mixture was extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated. Yield: 40.0 g (*S*)-amine **19**. ^{1}H NMR (360 MHz): δ = 7.16 (1H, ddd, J = 11.5, 7.7, 2.2 Hz), 7.10 (1H, dt, J = 10.1, 8.1 Hz), 6.99–7.05 (1H, m), 3.80 (1H, t, J = 6.8 Hz), 1.55–1.74 (2H, m), 1.50 (2H, brs), 0.85 ppm (3H, t, J = 7.4 Hz).

Methyl bromoacetate (26.5 mL) was added to a stirring solution of (*S*)-amine and (40 g) of DIPEA (81.2 mL) in THF (350 mL), and the reaction mixture was stirred for 5 days at room temperature. The resulting precipitate was filtered off, washed with THF, and then the filtrate was evaporated. The obtained residue was stirred in CH_2Cl_2 and then washed with a solution of $NaHCO_3$ and with H_2O. The organic layer was separated, dried ($MgSO_4$), filtered off, and then the solvent was evaporated and co-evaporated with toluene. Yield: 83%.

Formic acid (23 mL) was added to the residue of the previous reaction in xylene (400 mL). The reaction mixture was stirred and held at reflux for 4 h. The mixture was allowed to reach room temperature, and the solvent was evaporated. The residue was filtered over silica gel (eluent: CH_2Cl_2/CH_3OH 99:1). The desired product fractions were collected, the solvent was evaporated and co-evaporated with toluene. The residue was used without further purification.

The above residue in 500 mL THF was stirred and cooled to −78 °C, and 1,2-isoxazole-5-carboxyl chloride (49.3 g) was added. LiHMDS (400 mL, 80%) was added over a period of 30 min. The mixture was stirred for 4 h at −78 °C. The reaction temperature was then raised to −20 °C. A solution of HCl (66 mL, concd) in H_2O (400 mL) was added dropwise over 15 min. The organic solvent was evaporated. H_2O (400 mL) was added, followed by HCl (140 mL, concd), CH_3OH (800 mL), and KSCN (36 g). The reaction mixture was stirred for 22 h at 60 °C. H_2O was added, and the mixture was cooled to room temperature, then treated with K_2CO_3 and $NaHCO_3$. This mixture was extracted with CH_2Cl_2. The separated organic layer was dried, filtered, and the solvent was evaporated. The residue was purified by HPLC. The product fractions were collected, and the solvent was evaporated. The residue was crystallized from DIPE (2×). The precipitate was filtered off and dried. Yield: 23.4 g **24h**. ^{1}H NMR (360 MHz): δ = 11.9 (1H, brs), 8.40 (1H, d, J = 1.9 Hz), 7.00–7.23 (3H, m), 6.92 (1H, d, J = 2.0 Hz), 6.57 (1H, dd, J = 9.5, 6.2 Hz), 3.71 (3H, s), 2.38–2.61 (2H, m), 1.03 ppm (3H, t, J = 7.4 Hz); ^{13}C NMR (151 MHz, [D$_6$]DMSO): δ = 166.63, 158.37, 156.69, 151.41, 149.04 (dd, J = 245.0, 12.7 Hz), 148.35 (dd, J = 245.4, 12.5 Hz), 137.00 (t, J = 4.4 Hz), 123.49–123.53 (m), 121.36, 119.06, 117.09 (d, J = 17.6 Hz), 115.86 (d, J = 17.6 Hz), 105.27, 59.78, 52.37, 23.57, 10.76 ppm; mp: 127.2 °C; OR −172° (589 nm, $CHCl_3$, 20 °C); HRMS [M+H]$^+$ m/z calcd 380.0880, obsd 380.0885.

Syntheses of **24a**–**g** and **24i**–**x** were carried out by a similar procedure.

24a: ^{1}H NMR (360 MHz): δ = 11.45 (1H, brs), 7.42–7.50 (6H, m), 7.38 (1H, d, J = 8.4 Hz), 7.20 (1H, dd, J = 8.3, 2.2 Hz), 6.50 (1H, dd, J = 9.9, 6.0 Hz), 3.51 (3H, s), 2.51–2.65 (1H, m), 2.40–2.50 (1H, m), 1.01 ppm (3H, t, J = 7.5 Hz); HRMS [M+H]$^+$ m/z calcd 421.0544, obsd 421.0569.

24b: ^{1}H NMR (360 MHz): δ = 7.56 (1H, d, J = 3.8 Hz), 7.48 (1H, d, J = 5.1 Hz), 7.45 (1H, d, J = 2.1 Hz), 7.37 (1H, d, J = 8.4 Hz), 7.18 (1H, dd, J = 8.4, 2.1 Hz), 7.10 (1H, dd, J = 5.1, 3.8 Hz), 6.50 (1H, dd, J = 9.8, 6.0 Hz), 3.65 (3H, s), 2.50–2.61 (1H, m), 2.37–2.50 (1H, m), 1.01 ppm (3H, t, J = 7.4 Hz); HRMS [M+H]$^+$ m/z calcd 427.0109, obsd 427.0116.

24c: ^{1}H NMR (360 MHz): δ = 7.44 (1H, d, J = 2.2 Hz), 7.40 (1H, d, J = 8.4 Hz), 7.21 (1H, dd, J = 8.4, 2.2 Hz), 6.49 (1H, dd, J = 9.8, 6.1 Hz),

3.55 (3H, s), 2.70 (3H, s), 2.53–2.66 (1H, m), 2.40–2.52 (1H, m), 1.00 ppm (3H, t, $J=7.4$ Hz); HRMS $[M+H]^+$ *m/z* calcd 411.0449, obsd 411.0461.

24d: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=13.51$ (brs), 7.86 (dd, $J=1.8$, 0.8 Hz), 7.59 (d, $J=8.4$ Hz), 7.46 (dd, $J=2.2$, 1.0 Hz), 7.19 (ddd, $J=8.4$, 2.2, 1.0 Hz), 7.08 (dd, $J=3.5$, 0.8 Hz), 6.66 (dd, $J=3.5$, 1.8 Hz), 6.42 (dd, $J=10.2$, 5.7 Hz), 3.60 (s), 2.38–2.47 (m), 2.28–2.40 (m), 0.84 ppm (t, $J=7.3$ Hz); HRMS $[M+H]^+$ *m/z* calcd 411.0259, obsd 411.0261.

24e: ^{1}H NMR (360 MHz, [D$_6$]DMSO): $\delta=13.92$ (1H, brs), 8.76 (1H, d, $J=1.9$ Hz), 7.60 (1H, d, $J=8.4$ Hz), 7.49 (1H, d, $J=2.2$ Hz), 7.21 (1H, dd, $J=8.4$, 2.2 Hz), 7.01 (1H, d, $J=1.9$ Hz), 6.43 (1H, dd, $J=10.1$, 5.7 Hz), 3.63 (3H, s), 2.39–2.50 (1H, m), 2.36 (1H, brs), 0.85 ppm (3H, t, $J=7.3$ Hz); HRMS $[M+H]^+$ *m/z* calcd 412.0289, obsd 412.0292.

24f: ^{1}H NMR (360 MHz, [D$_6$]DMSO): $\delta=13.92$ (1H, brs), 8.76 (1H, d, $J=1.9$ Hz), 7.60 (1H, d, $J=8.4$ Hz), 7.49 (1H, d, $J=2.2$ Hz), 7.21 (1H, dd, $J=8.4$, 2.2 Hz), 7.01 (1H, d, $J=1.9$ Hz), 6.43 (1H, dd, $J=10.1$, 5.7 Hz), 3.63 (3H, s), 2.39–2.50 (1H, m), 2.36 (1H, brs), 0.85 ppm (3H, t, $J=7.3$ Hz); OR -158.84° (589 nm, $CHCl_3$, 20 °C); HRMS $[M+H]^+$ *m/z* calcd 412.0289, obsd 412.0290.

24g: ^{1}H NMR (360 MHz): $\delta=11.21$ (1H, brs), 8.39 (1H, d, $J=2.0$ Hz), 7.07–7.20 (2H, m), 7.01–7.07 (1H, m), 6.93 (1H, d, $J=1.9$ Hz), 6.57 (1H, t, $J=7.9$ Hz), 3.71 (3H, s), 2.38–2.57 (2H, m), 1.03 ppm (3H, t, $J=7.4$ Hz); mp: 135 °C; HRMS $[M+H]^+$ *m/z* calcd 380.0880, obsd 380.0894

24i: ^{1}H NMR (360 MHz): $\delta=2.18$ (1H, brs), 8.40 (1H, d, $J=1.9$ Hz), 7.00–7.23 (3H, m), 6.92 (1H, d, $J=2.0$ Hz), 6.57 (1H, dd, $J=9.5$, 6.2 Hz), 3.71 (3H, s), 2.38–2.61 (2H, m), 1.03 ppm (3H, t, $J=7.4$ Hz); mp: 120 °C; OR $+170^\circ$ (589 nm, $CHCl_3$, 20 °C); HRMS $[M+H]^+$ *m/z* calcd 380.0880, obsd 380.0892.

24j: ^{1}H NMR (360 MHz): $\delta=10.29$ (1H, brs), 7.41 (1H, d, $J=2.2$ Hz), 7.39 (1H, d, $J=8.4$ Hz), 7.16 (1H, dd, $J=8.4$, 2.2 Hz), 6.56 (1H, t, $J=8.0$ Hz), 6.47 (1H, s), 3.67 (3H, s), 2.49 (3H, s), 2.40–2.48 (2H, m), 1.02 ppm (3H, t, $J=7.4$ Hz); HRMS $[M+H]^+$ *m/z* calcd 426.0446, obsd 426.0462.

24k: ^{1}H NMR (360 MHz): $\delta=10.06$ (1H, brs), 7.03–7.22 (3H, m), 6.56 (1H, t, $J=7.9$ Hz), 6.42 (1H, s), 3.66 (3H, s), 2.48 (3H, s), 2.35–2.55 (2H, m), 1.03 ppm (3H, t, $J=7.3$ Hz); mp: 58.1 °C; HRMS $[M+H]^+$ calcd 394.1037, obsd 394.1037.

24l: ^{1}H NMR (360 MHz): $\delta=12.44$ (1H, brs), 7.04–7.23 (3H, m), 6.50 (1H, dd, $J=10.0$, 5.9 Hz), 3.57 (3H, s), 2.56–2.68 (1H, m), 2.38–2.51 (1H, m), 2.36 (3H, s), 2.18 (3H, s), 0.97 ppm (3H, t, $J=7.3$ Hz); HRMS $[M+H]^+$ *m/z* calcd 408.1193, obsd 408.1202.

24m: ^{1}H NMR (360 MHz): $\delta=10.89$ (1H, brs), 8.63 (1H, d, $J=4.8$ Hz), 7.87 (1H, d, $J=8.1$ Hz), 7.75 (1H, td, $J=7.8$, 1.8 Hz), 7.44 (1H, d, $J=2.2$ Hz), 7.39 (1H, d, $J=8.4$ Hz), 7.31 (1H, dd, $J=7.6$, 4.8 Hz), 7.19 (1H, dd, $J=8.4$, 2.2 Hz), 6.53 (1H, t, $J=7.9$ Hz), 3.63 (3H, s), 2.34–2.50 (2H, m), 1.05 ppm (3H, t, $J=7.4$ Hz); mp: 188 °C; HRMS $[M+H]^+$ *m/z* calcd 422.0497, obsd 422.0478.

24n: ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.69$ (1H, d, $J=2.3$ Hz), 8.57 (1H, d, $J=4.8$ Hz), 7.94 (1H, dt, $J=7.9$, 2.0 Hz), 7.57 (1H, d, $J=8.4$ Hz), 7.48 (1H, s), 7.44 (1H, dd, $J=7.9$, 4.8 Hz), 7.23 (1H, dd, $J=8.4$, 2.2 Hz), 6.49–6.61 (1H, m), 3.41 (3H, s), 2.36–2.46 (2H, m), 0.83 ppm (3H, t, $J=7.3$ Hz); HRMS $[M+H]^+$ *m/z* calcd 422.0497, obsd 422.0481.

24o: ^{1}H NMR (360 MHz): $\delta=12.98$ (1H, brs), 8.70 (2H, d, $J=6.2$ Hz), 7.46 (1H, d, $J=2.1$ Hz), 7.42 (2H, d, $J=6.2$ Hz), 7.39 (1H, d, $J=8.4$ Hz), 7.20 (1H, dd, $J=8.4$, 2.2 Hz), 6.49 (1H, dd, $J=9.7$, 6.2 Hz), 3.55 (3H, s), 2.36–2.66 (2H, m), 1.01 ppm (3H, t, $J=7.3$ Hz); mp: 169 °C; HRMS $[M+H]^+$ *m/z* calcd 422.0497, obsd 422.0491.

24p: ^{1}H NMR (360 MHz): $\delta=11.06$ (1H, brs), 9.10 (1H, d, $J=1.6$ Hz), 8.63 (1H, t, $J=2.1$ Hz), 8.60 (1H, d, $J=2.6$ Hz), 7.44 (1H, d, $J=2.2$ Hz), 7.40 (1H, d, $J=8.4$ Hz), 7.19 (1H, dd, $J=8.4$, 2.2 Hz), 6.54 (1H, t, $J=7.9$ Hz), 3.68 (3H, s), 2.47 (1H, t, $J=7.5$ Hz), 1.05 ppm (3H, t, $J=7.4$ Hz); mp: 201.6 °C; HRMS $[M+H]^+$ *m/z* calcd 423.0449, obsd 423.0455.

24q: ^{1}H NMR (360 MHz): $\delta=10.27$ (1H, brs), 9.10 (1H, d, $J=1.6$ Hz), 8.59 (1H, d, $J=2.6$ Hz), 8.61 (1H, dd, $J=2.6$, 1.6 Hz), 7.03–7.22 (3H, m), 6.54 (1H, t, $J=8.0$ Hz), 3.68 (3H, s), 2.43–2.52 (2H, m), 1.06 ppm (3H, t, $J=7.4$ Hz); HRMS $[M+H]^+$ *m/z* calcd 391.1040, obsd 391.1058.

24r: ^{1}H NMR (360 MHz): $\delta=1.73$ (1H, brs), 7.44 (1H, d, $J=2.2$ Hz), 7.39 (1H, d, $J=8.4$ Hz), 7.21 (1H, dd, $J=8.4$, 2.2 Hz), 6.53 (1H, dd, $J=10.0$, 6.0 Hz), 6.22 (1H, s), 3.66 (3H, s), 3.56 (3H, s), 2.53–2.68 (1H, m), 2.39–2.53 (1H, m), 2.28 (3H, s), 0.99 ppm (3H, t, $J=7.4$ Hz); mp: 148 °C; HRMS $[M+H]^+$ calcd 439.0762, obsd 439.0766.

24s: ^{1}H NMR (360 MHz): $\delta=11.11$ (1H, brs), 7.45 (1H, d, $J=2.2$ Hz), 7.39 (1H, d, $J=8.4$ Hz), 7.22 (1H, dd, $J=8.4$, 2.2 Hz), 6.53 (1H, dd, $J=9.9$, 6.0 Hz), 6.21 (1H, s), 3.66 (3H, s), 3.56 (3H, s), 2.61 (1H, brs), 2.40–2.52 (1H, m), 2.28 (3H, s), 1.00 ppm (3H, t, $J=7.4$ Hz); HRMS $[M+H]^+$ calcd 439.0762, obsd 439.0759.

24t: ^{1}H NMR (360 MHz): $\delta=7.45$ (1H, d, $J=2.1$ Hz), 7.36 (1H, d, $J=8.4$ Hz), 7.19 (1H, dd, $J=8.4$, 2.2 Hz), 7.13 (1H, d, $J=1.3$ Hz), 7.01 (1H, d, $J=1.2$ Hz), 6.64 (1H, dd, $J=9.4$, 6.5 Hz), 3.56 (3H, s), 3.53 (3H, s), 2.38–2.61 (2H, m), 1.01 ppm (3H, t, $J=7.4$ Hz); mp: 165 °C; HRMS $[M+H]^+$ *m/z* calcd 425.0606, obsd 425.0613.

24u: ^{1}H NMR (360 MHz): $\delta=10.53$ (1H, s), 7.42 (1H, brd, $J=6.3$ Hz), 7.36 (0.5H, d, $J=8.4$ Hz), 7.36 (0.5H, d, $J=8.4$ Hz), 7.18 (1H, brt, $J=8.3$ Hz), 6.56 (1H, dd, $J=10.0$, 5.9 Hz), 5.21 (1H, td, $J=7.3$, 5.4 Hz), 4.13 (1H, dt, $J=8.4$, 6.4 Hz), 3.85–3.92 (1H, m, $J=8.3$, 7.0, 7.0, 1.3 Hz), 3.69 (1.5H, s), 3.68 (1.5H, s), 2.57 (1H, brs), 2.34–2.52 (2H, m), 1.93–2.07 (2H, m), 1.71–1.87 (1H, m), 0.94 (1.5H, t, $J=7.4$ Hz), 0.94 ppm (1.5H, t, $J=7.4$ Hz); mp: 184 °C; HRMS $[M+H]^+$ *m/z* calcd 415.0650, obsd 415.0651.

24v: ^{1}H NMR (360 MHz): $\delta=10.42$ (1H, brs), 7.40 (1H, s), 7.36 (1H, d, $J=8.4$ Hz), 7.17 (1H, d, $J=8.4$ Hz), 6.55 (1H, dd, $J=9.7$, 6.2 Hz), 4.05–4.17 (2H, m), 3.93 (1H, td, $J=8.8$, 3.3 Hz), 3.79–3.87 (2H, m), 3.69 (3H, s), 2.50 (1H, brs), 2.34–2.47 (2H, m), 1.94–2.04 (1H, m), 0.94 ppm (3H, t, $J=7.3$ Hz); mp: 181 °C; HRMS $[M+H]^+$ *m/z* calcd 415.0650, obsd 415.0648.

24w: ^{1}H NMR (400 MHz): $\delta=7.40$ (1H, d, $J=2.2$ Hz), 7.34 (1H, d, $J=8.4$ Hz), 7.15 (1H, dd, $J=8.4$, 2.2 Hz), 6.54 (1H, dd, $J=9.7$, 6.1 Hz), 5.94 (1H, brs), 3.66 (3H, s), 3.15–3.26 (1H, m), 2.99 (2H, dt, $J=11.8$, 2.9 Hz), 2.34–2.58 (2H, m), 2.31 (3H, s), 1.98–2.09 (2H, m), 1.78–1.89 (4H, m), 0.95 ppm (3H, t, $J=7.4$ Hz); mp: 192 °C; HRMS $[M+H]^+$ *m/z* calcd 442.1123, obsd 442.1132.

24x: ^{1}H NMR (400 MHz): $\delta=7.41$ (1H, d, $J=2.2$ Hz), 7.35 (1H, d, $J=8.4$ Hz), 7.18 (1H, dd, $J=8.4$, 2.2 Hz), 6.60 (1H, t, $J=7.9$ Hz), 3.65 (3H, s), 3.54 (1H, brs), 2.91–3.01 (2H, m), 2.37–2.48 (2H, m), 2.31 (4H, brs), 1.96–2.07 (1H, m), 1.75–1.86 (1H, m), 1.52–1.73 (3H, m), 0.91 ppm (3H, t, $J=7.4$ Hz); mp: 182 °C; HRMS $[M+H]^+$ *m/z* calcd 442.1123, obsd 442.1109.

Synthesis of 27a: 4 (18.9 g) and dimethyloxalate (18.6 g) in THF (100 mL) were treated with NaOMe (10.34 g) and stirred for 24 h. The solvent was evaporated, the residue was dissolved in H_2O and

washed with ether. CH_3OH (200 mL) and KSCN (10 g) were added to the separated aqueous layer, and the reaction mixture was stirred for 18 h at 80 °C. The solvent was partly evaporated, and the precipitate was filtered and purified over silica gel (eluent: CH_2Cl_2/CH_3OH 98:2). After evaporation of the fractions containing the product, the compound was triturated with hexanes, filtered, and dried in vacuo; 5.2 g **1a** were obtained. ^{1}H NMR (360 MHz): $\delta = 11.01$ (1H, brs), 7.43 (1H, d, $J = 2.2$ Hz), 7.41 (1H, d, $J = 8.4$ Hz), 7.17 (1H, dd, $J = 8.4$, 2.2 Hz), 6.28 (1H, t, $J = 8.0$ Hz), 3.86 (3H, s), 3.65 (3H, s), 2.29–2.41 (2H, m, $J = 7.5$, 7.5, 7.5, 7.5 Hz), 1.05 ppm (3H, t, $J = 7.4$ Hz).

1a (200 mg) was stirred at room temperature in 1 N NaOH (25 mL) until dissolution. The mixture was neutralized with 1 N HCl, and the precipitate was filtered and dried in vacuo to give 110 mg **26**. ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 13.80$ (1H, brs), 13.43 (1H, brs), 7.62 (1H, d, $J = 8.4$ Hz), 7.45 (1H, d, $J = 2.2$ Hz), 7.21 (1H, dd, $J = 8.4$, 2.2 Hz), 6.23 (1H, dd, $J = 10.1$, 6.1 Hz), 3.60 (3H, s), 2.26–2.38 (1H, m), 2.12–2.24 (1H, m), 0.87 ppm (3H, t, $J = 7.2$ Hz); mp: 182 °C.

A mixture of **26** (100 mg) in thionyl chloride (5 mL) was stirred overnight at room temperature, the solvent was evaporated and co-evaporated with toluene. The resulting residue was used as such in the next reaction step. Acetyl hydrazine (1 g) was added in one portion to a solution of the residue obtained above in THF (10 mL) under N_2, and the reaction mixture was stirred for 1 h. The solvent was evaporated, the residue was taken up in CH_2Cl_2 and washed with H_2O and HCl (1 N). The organic layer was separated, dried ($MgSO_4$), filtered off, and the solvent was evaporated, then co-evaporated with toluene. Yield: 0.11 g of the acetyl hydrazide.

The acetyl hydrazide (102 mg) obtained above in phosphoryl chloride (5 mL) was stirred at 60 °C for 18 h, and then the reaction mixture was allowed to reach room temperature. The solvent was evaporated, and the residue was quenched with iced H_2O. The mixture was extracted with CH_2Cl_2, and the organic layer was separated, then evaporated. The residue was dissolved in acetone (5 mL) and treated with SO_2 (g) for 10 min. The solvent was evaporated, and the residue was purified by RP HPLC. The product fractions were collected, and the organic volatiles were evaporated. The product was extracted with CH_2Cl_2, and the organic layer was separated, dried ($MgSO_4$), filtered off, and evaporated. Yield: 0.0276 g **27a**. ^{1}H NMR (360 MHz): $\delta = 12.05$ (1H, brs), 7.45 (1H, d, $J = 2.2$ Hz), 7.40 (1H, d, $J = 8.4$ Hz), 7.20 (1H, dd, $J = 8.4$, 2.2 Hz), 6.48 (1H, dd, $J = 9.4$, 6.6 Hz), 3.72 (3H, s), 2.61 (3H, s), 2.37–2.60 (2H, m), 1.04 ppm (3H, t, $J = 7.4$ Hz); mp: 154 °C; HRMS $[M+H]^+$ *m/z* calcd 427.0398, obsd 427.0399.

27b was synthesized by a similar procedure. ^{1}H NMR (360 MHz, [D$_6$]DMSO): $\delta = 14.13$ (1H, brs), 7.40 (1H, dt, $J = 10.7$, 8.6 Hz), 7.32 (1H, ddd, $J = 12.2$, 7.7, 2.3 Hz), 7.01–7.09 (1H, m), 6.38 (1H, dd, $J = 9.9$, 6.0 Hz), 3.60 (3H, s), 2.57 (3H, s), 2.27–2.48 (2H, m), 0.86 ppm (3H, t, $J = 7.3$ Hz); mp: 142 °C; HRMS $[M+H]^+$ *m/z* calcd 395.0989, obsd 395.1002.

Calcium mobilization assays

Human monocytic THP-1 cells were loaded with 4 μM Fluo-3AM (Invitrogen) for 30 min at 37 °C in RPMI-1640 medium containing HEPES (20 mM), bovine serum albumin (BSA, 0.1%), and probenecid (5 mM). Cells were washed and further incubated in calcium buffer containing HEPES (5 mM), NaCl (140 mM), $MgCl_2$ (1 mM), KCl (5 mM), glucose (10 mM), probenecid (2.5 mM), $CaCl_2$ (1.25 mM), and BSA (0.1%). Compounds were dissolved in DMSO to 100× dilutions, and further diluted in calcium buffer (final [DSMO]: 1%). Cells were pre-incubated with test compounds for 20 min at room temperature before human recombinant MCP-1 was added (R&D Systems). Changes in intracellular free $[Ca^{2+}]$ were measured using the Fluorescent Imaging Plate Reader (Molecular Devices). IC_{50} values were calculated using GraphPad Prism software.

Chemotaxis

Peripheral blood mononuclear cells (PBMC) from heparinized human blood were isolated using Ficoll–Paque gradient centrifugation (Amersham Biosciences). Assays of chemotactic responsiveness were performed using disposable 96-well chemotaxis chambers with polycarbonate filter membranes (5 μm, ChemoTx, Neuro Probe). PBMC were fluorescently labeled with Calcein-AM (5 μg mL^{-1}, Invitrogen) containing Pluronic F-127 (0.05%, Invitrogen) at 37 °C for 30 min. Labeled cells were resuspended in HBSS (Gibco BRL) supplemented with BSA (0.2%). Subsequently, cells were pre-incubated for 10 min at room temperature with serial dilutions of the compounds (final [DSMO]: 0.2%), before adding to the topside of the filter. Migration to MCP-1 (30 ng mL^{-1}) in the bottom wells was permitted for 2 h at 37 °C; non-migrated cells were then removed from the top of the filter by gently wiping. The migrated cells were measured using a fluorescent plate reader ($\lambda_{ex} = 485$ nm; $\lambda_{em} = 538$ nm).

Hepatic metabolic stability assays

Hepatic metabolic stability was determined as described by Kantharaj et al.[18] Briefly, all incubations were conducted by shaking reaction mixtures (250 μL) in duplicate containing test compound (5 μM), microsomal protein (1 mg mL^{-1}), phosphate buffer (0.5 M, pH 7.4), $MgCl_2$ (1.6 mM), glucose-6-phosphate (1.6 mM), and glucose-6-phosphate-dehydrogenase (0.125 U mL^{-1}). The mixtures were pre-incubated for 5 min at 37 °C, followed by the addition of NADP (0.16 mM) to initiate the biochemical reaction. Reactions were terminated after 15 min by addition of 2 volumes of DMSO. The precipitated material was removed by centrifugation, and the supernatant was analyzed by LC–MS on a ThermoFinnigan Ion Trap instrument. Metabolic stability was determined by comparing the peak areas of the parent compound measured at 15 min with that at 0 min. For cases in which the areas decreased at T_0 due to esterase, amidase, or reductase activities, metabolic stability was calculated using the appropriate control (T_{-5} boiled enzyme).

Plasma stability assays

Pooled ($n = 8$) mouse and human plasma ($n = 10$) were obtained from Bioreclamatrin (Hicksville, NY, USA). Compounds were dissolved in DMSO and spiked to plasma at a concentration of 2000 ng mL^{-1} (in 250 μL). Compounds were thereafter incubated for 2 h at 37 °C, and the reaction was terminated by the addition of 2 volumes of DMSO followed by centrifugation for protein precipitation. Supernatants were analyzed for their remaining concentration of parent compound. Compounds were classified as instable if >20% of the parent compound was converted.

Ethical disclaimer

The experimental protocols were approved by the ethical committee for animal experimentation of Janssen Pharmaceutica NV and adhere to the *Principles of Laboratory Animal Care* published by the National Institutes of Health (NIH, 1985).

Rat in vivo determination of PK parameters

Male SPF Sprague–Dawley rats, each weighing ~250 g, were used. Compounds were dissolved in pyrogen-free water, tartaric acid was added for total dissolution of the compounds, and the solution was adjusted to pH 4.4 with NaOH. Before dosing, the formulations were stored at room temperature, protected from light, and analyzed quantitatively; the stability of the formulations was checked at the day of dosing. Oral dose (10 mg kg^{-1}) administration was by gastric intubation, and blood samples were collected at 0.5, 1, 2, 4, 8, and 24 h post administration. Intravenous dose administration (2.5 mg kg^{-1}) was in the jugular vein, and blood samples were collected at 7 and 20 min, and 1, 2, 4, 8, and 24 h post administration. From three different animals the complete plasma profiles were taken. A limited PK analysis was performed using WinNonlin™ Professional (Version 3.3, Pharsight, Mountain View, CA, USA). Analysis was carried out on the plasma concentration time profiles obtained from each animal ($n=3$). Oral PK parameters observed were maximum plasma concentration (C_{max}), time to reach the maximum plasma concentration (T_{max}), plasma half-life ($t_{1/2}$), and exposure of the compound calculated by the area under the curve (AUC_{last} and AUC_{inf}). PK parameters after i.v. administration were volume of distribution (V_d), total plasma clearance (Cl) and area under the curve (AUC_{last} and AUC_{inf}).

Keywords: bioisosteres · CCR2 · inhibitors · MCP-1 · thioimidazoles

[1] M. K. Schwartz, T. N. C. Wells, *Nat. Rev. Drug Discovery* **2002**, *1*, 347.
[2] J. J. Onuffer, R. Horuk, *Trends Pharmacol. Sci.* **2002**, *23*, 459.
[3] J. Dawson, W. Miltz, A. K. Mir, C. Wiessner, *Expert Opin. Ther. Targets* **2003**, *7*, 35.
[4] M. Feria, F. Díaz-González, *Expert Opin. Ther. Pat.* **2006**, *16*, 49.
[5] M. Baba, O. Nishimura, N. Kanzaki, M. Okamoto, H. Sawada, Y. Iizawa, M. Shiraishi, Y. Aramaki, K. Okonogi, Y. Ogawa, K. Meguro, M. Fujino, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5698.
[6] M. Shiraishi, Y. Aramaki, M. Seto, H. Imoto, Y. Nishikawa, N. Kanzaki, M. Okamoto, H. Sawada, O. Nishimura, M. Baba, M. Fujino, *J. Med. Chem.* **2000**, *43*, 2049.
[7] I. T. Forbes, D. G. Cooper, E. K. Dodds, D. M. Hickey, R. J. Ife, M. Meeson, M. Stockley, T. A. Berkhout, J. Gohil, P. H. Groot, K. Moores, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1803.
[8] J. Witherington, V. Bordas, D. G. Cooper, I. T. Forbes, A. D. Gribble, R. J. Ife, T. Berkhout, J. Gohil, P. H. Groot, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2177.
[9] T. Mirzadegan, F. Diehl, B. Ebi, S. Bhakta, I. Polsky, D. McCarley, M. Mulkins, G. S. Weatherhead, J. M. Lapierre, J. Dankwardt, D. Morgans, Jr., R. Wilhelm, K. Jarnagin, *J. Biol. Chem.* **2000**, *275*, 25562.
[10] W. J. Moree, K. Kataoka, M. M. Ramirez-Weinhouse, T. Shiota, M. Imai, M. Sudo, T. Tsutsumi, N. Endo, Y. Muroga, T. Hada, H. Tanaka, T. Morita, J. Greene, D. Barnum, J. Saunders, Y. Kato, P. L. Myers, C. M. Tarby, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5413.
[11] L. Yang, C. Zhou, L. Guo, G. Morriello, G. Butora, A. Pasternak, W. H. Parsons, S. G. Mills, M. MacCoss, P. P. Vicario, H. Zweerink, M. J. Ayala, S. Goyal, W. A. Hanlon, M. A. Cascieri, M. S. Springer, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3735.
[12] B. Lagu, C. Gerchak, M. Pan, C. Hou, M. Singer, R. Malaviya, M. Matheis, G. Olini, D. Cavender, M. Wachter, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4382.
[13] Meeting report: *7th Annual European Congress of Rheumatology*, June 2006, Amsterdam, The Netherlands: F. Cameron, S. Crofskey, *Int. J. Pharm. Med.* **2006**, *20*, 321.
[14] *Abstract of the EULAR Meeting*, June 21–23, 2006, Amsterdam, The Netherlands: C. E. Vergunst, D. M. Gerlag, L. Lopatinskaya, L. Klareskog, M. D. Smith, D. Baeten, P. P. Tak; Poster no. 489.
[15] J. Haringman, D. Gerlag, T. Smeets, D. Baeten, F Van den Bosch, B. Bresnihan, F. Breedveld, H. Dinant, F. Legay, H. Gram, P. Loetscher, R. Schmouder, T. Woorworth, P. Tak, *Arthritis Rheum.* **2006**, *54*, 2387.
[16] G. Van Lommen, J. Doyon, E. Coesemans, S. Boeckx, M. Cools, M. Buntinx, B. Hermans, J. Van Wauwe, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 497.
[17] T. Kuppens, W. Heerbout, B. Van der Veken, D. Corens, A. H. J. De Groot, J. Doyon, G. Van Lommen, P. Bultinck, *Chirality* **2006**, *18*, 609.
[18] E. Kantharaj, A. Tuytelaars, P. Proost, Z. Ongel, H. van Assouw, R. Gilissen, *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2661.

Received: October 2, 2007
Revised: November 30, 2007
Published online on January 10, 2008

