β-Aryl-Succinic Acid Hydroxamates as Dual Inhibitors of Matrix Metalloproteinases and Tumor Necrosis Factor Alpha Converting Enzyme

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Novel hydroxamate inhibitors of tumor necrosis factor converting enzyme (TACE) and matrix metalloproteases (MMPs) have been synthesized via the Claisen−Ireland rearrangement. Aryl residues have been introduced to fill the enzyme's P1′ specificity pocket. The best compound inhibits MMPs and TACE with nanomolar potency and inhibits the release of TNFα from cells with an IC_{50} of 48 nM. Oral administration to rats inhibits the LPS-induced plasma TNFα levels with an ED_{50} of 1 mg/kg.

Introduction

Rheumatoid arthritis (RA) is a chronic, inflammatory joint disease which affects about 1% of the population worldwide. Clinical signs include pain and joint swelling, and over the long term, the erosion of cartilage and bone leads to irreversible destruction of the affected joints. The cytokines tumor necrosis factor α (TNFα) and interleukin-1*β* (IL-1*β*) are well-established mediators of inflammation and joint destruction.[1] Clinical studies and preclinical models suggest a central early role of TNFα which seems to orchestrate the network of pro-inflammatory cytokines.[2] Antagonizing the TNFα by the soluble TNF receptor (Enbrel) has been proven to be effective for the treatment of RA.[3]

The degradation of cartilage and bone collagen in RA is accomplished by matrix metalloproteinases (MMPs), produced by synovial fibroblasts, chondrocytes, and infiltrating leukocytes.[4] Several different MMPs have been shown to be present in the joints of RA patients.[5]

Attacking the inflammatory as well as the erosive component of RA with one therapeutic agent might be possible by designing dual inhibitors of MMPs and TNFα convertase (TACE), a metalloproteinase responsible for the release of TNFα from its precursor, proTNFα.[6,7] MMPs and TACE are Zn-dependent neutral metalloproteinases; the enzymes share considerable structural homology in the active-site region as shown by X-ray crystallography.[8] There is a vast amount of literature on inhibitors of MMPs containing hydroxamates, thiols, carboxylates, or phosphinates as the Zn-chelating group,[9] and dual inhibition of MMPs and TNFα release was described for some MMP inhibitors.[10] Very recently, the reversed hydroxamate dual MMP/TACE inhibitor GW3333 has been described.[11]

Many of the MMP/TACE inhibitors reported to date are derived from marimastat (**1**), the first MMP inhibitor which entered clinical trials for cancer treatment.[12] Despite their nanomolar potency on MMPs, many of the succinate-based hydroxamate MMP inhibitors only modestly inhibit cellular TNFα release. TACE inhibitory activity in the marimastat template was improved by derivatization of the position α to the hydroxamate, leading to sub-micromolar inhibition of cellular TNFα release.[13] The vast majority of variations in the *β*-position, due to synthetic restrictions, focused on alkyl and aryl−alkyl residues that did not improve the TACE inhibitory activity over that of marimastat.[14,15]

In an attempt to improve the potency on TNFα inhibition and to overcome the poor in vivo properties of succinate-based hydroxamate MMP/TACE inhibitors, we have introduced novel modifications in α- and *β*-positions of the succinate hydroxamate template via a Claisen−Ireland rearrangement.[16] This methodology was used to place aryl substituents in the position *β* to the hydroxamate, which resulted in a series (Figure 1) of novel and very potent dual inhibitors of MMPs and TACE as well as cellular TNFα release.

Chemistry

The succinate inhibitors **2**−**5** presented in this study were synthesized by applying a Claisen−Ireland rearrangement[17] to build the succinate template and to introduce novel substituents R1 and R2. Scheme 1 describes the synthesis of compound **5** in detail. Compounds **2**−**4** were prepared in analogy to the described synthesis; however, the syntheses were carried through without separating the diastereomeric mixtures obtained after the Claisen−Ireland rearrangement. Separation of the diastereomers was done at the end of the synthesis via reverse-phase HPLC.

trans-Dibromobut-2-ene **6** was reacted with benzyl alcohol under phase-transfer catalysis to give the benzyl ether **7**. DBU-mediated coupling of **7** with (4-methoxyphenyl)acetic acid yielded the allyl ester **8**. The Claisen−Ireland rearrangement of **8** was initiated with Li-HMDS and TMS-Cl in the presence of catalytic amounts of $TiCl_4$ and resulted in the succinate precursor **9**, which was isolated as a mixture of 4 diastereomers. The anti-isomer **10** was obtained enantiomerically pure via crystallization of **9** as its (−)-1-(*S*)-phenylethylamine salt and subsequent acidification. Reaction of **10** with *tert*-leucine methylamide under EDCI/HOBT conditions and oxidation of the resulting coupling product **11** in a three-step process gave the succinate derivative **12**. Conversion of the acid **12** to the hydroxamic acid inhibitor **5** was achieved by coupling with *o*-benzylhy-

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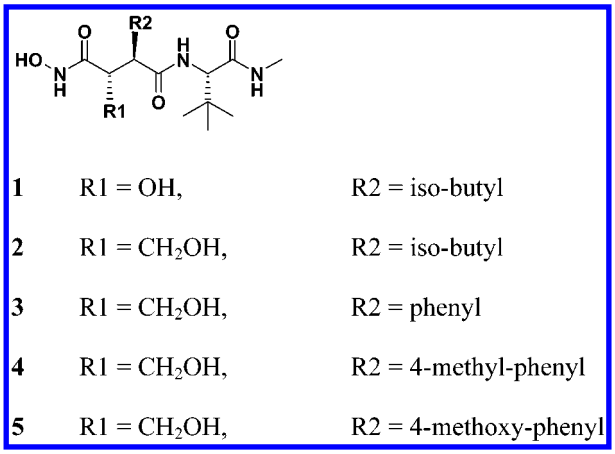


1	R1 = OH,	R2 = iso-butyl
2	R1 = CH_2OH,	R2 = iso-butyl
3	R1 = CH_2OH,	R2 = phenyl
4	R1 = CH_2OH,	R2 = 4-methyl-phenyl
5	R1 = CH_2OH,	R2 = 4-methoxy-phenyl

Figure 1. Structures of novel MMP/TACE inhibitors.

droxylamine and EDCI to **13** and removal of the protection groups via Pd-$BaSO_4$-catalyzed hydrogenation. Proof of the absolute stereochemistry of the succinate inhibitor **5** was established via X-ray crystallography.

Discussion

The inhibitory potency of the succinate inhibitors was determined against recombinant TACE, MMP-1, MMP-2, and MMP-3 using a fluorogenic substrate. TNFα inhibition in cells was measured in LPS-stimulated human mononuclear cells (hPBMCs). The results are summarized in Table 1.

The use of the Claisen−Ireland rearrangement resulted in a hydroxymethylene substituent in the position α to the hydroxamate instead of a hydroxy group like in marimastat (**1**). As seen with compound **2** in Table 1, this modification retained the marimastat-like potency. While the activity of **2** on the MMPs was similar to that of marimastat, a 2-fold loss in activity on TACE as well as in the cellular TNFα release assay was found. Replacement of the isobutyl side chain in **2** by a phenyl group restored the inhibitory potency on TACE and TNFα release as illustrated by **3** but was less suitable for MMP activity. A drop in activity on all three MMPs was observed. Further improvements in potency on TACE and TNFα release were achieved by introduction of *para*-substituents in **3**. The *para*-methyl inhibitor **4** had similar activity on TACE compared to the phenyl analogue **3**, but the IC_{50} for cellular TNFα release decreased by more than a factor of 3. While the potency on MMP-2 increased 10-fold, the activity on MMP-1 and MMP-3 was hardly influenced by this modification. Changing the *para*-methyl group in **4** to a methoxy group (e.g., **5**) gave an additional increase in potency on TACE and cellular TNFα release and also improved the activity on MMP-1 and, to a greater extent, on MMP-3. Compared to marimastat, the *para*-methoxy compound **5** is a very potent inhibitor of TACE and cellular TNFα release as well as a broad spectrum MMP inhibitor with sub-nanomolar activity on all three enzymes tested. The solubility of compounds **2**−**5** in water is >10 mg/mL, and they show little or no binding to plasma proteins (data not shown).

The in vivo activity of compounds **1** and **3**−**5** was demonstrated in an LPS-induced systemic TNFα release model in rats. Four hours after oral dosing, the animals were challenged by an iv LPS injection and bled 1 h later. Concentrations of TNFα in rat plasma were determined, and a dose-dependent response was observed with all compounds tested. The starting compound marimastat (**1**) only showed statistically significant inhibition (87%) at the 30 mg/kg dose. In contrast, compounds **3**−**5** already had significant effects at much lower doses (**3**, 57% at 10 mg/kg; **5**, 62 and 79% at 3 and 10 mg/kg, respectively), with **5** being the most potent compound in the test with an ED_{50} of 1 mg/kg.

Conclusion

The application of the Claisen−Ireland rearrangement for the synthesis of succinate-based hydroxamate TACE/MMP inhibitors provides a convenient route for the introduction of aryl substituents in the position β to the hydroxamate. Compared to marimastat, the replacement of the isobutyl residue by *para*-substituted phenyl groups has led to improved potency against both TACE and cellular TNFα release. The *para*-methoxy derivative **5** was found to be a sub-nanomolar inhibitor of TACE with nanomolar activity in the cellular TNFα release assay as well as a sub-nanomolar inhibitor against MMP-1, MMP-2, and MMP-3. The in vivo activities of **5** in the LPS rat model justified proposing **5** for testing in chronic models and, possibly, as a candidate for further development.

Experimental Section

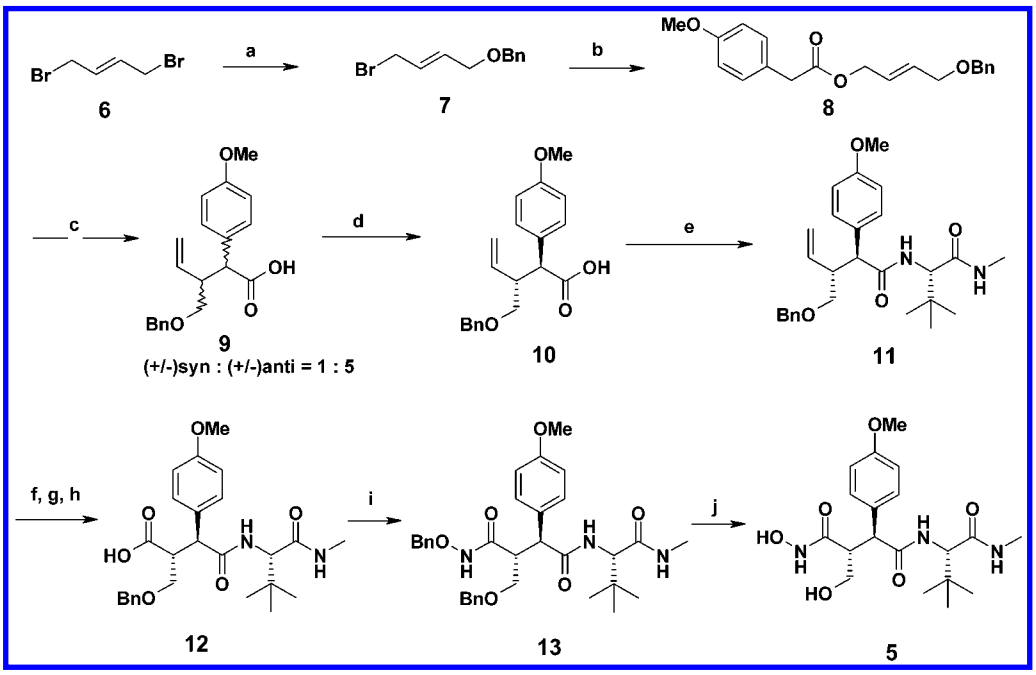
MMP Assays. In vitro inhibition of MMP-1 and -2 was measured using the fluorescence-quenched peptide substrate Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (5 μM) and a MMP concentration of 0.1 nM in 0.1 M Tris-HCl, containing 0.1 M NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, and 0.1% PEG6000, at 25 °C and pH 7.5. Inhibitors (11 concentrations), enzyme, and buffer were incubated in a black 96-well plate for 1 h. The reaction was started by the addition of substrate, and fluorescence (325/400 nm) was measured for 20 min in a Spectramax Gemini plate reader (Molecular Devices). The rates were fitted to the equation $v = v_o/(1 + [I]/K_i)$ where v and v_o are the rates in the presence and absence of inhibitor, respectively, and K_i is the dissociation constant. The substrate concentration of 5 μM is $\ll K_M$ for all MMPs, and no correction for substrate competition is needed. MMP-3 was assayed using Ac-Pro-Leu-Ala(S)-Nvl-Trp-NH_2 and DTNB in 50 mM MES and 10 mM $CaCl_2$ at pH 6.5.[18] Inhibitors and the enzyme were preincubated for 3 h, and the reaction was followed at 410 nm in a BioTec Elx808 plate reader for 40 min.

TACE Assay. The cDNA for human TACE (Met 1 to Asp 670) was cloned from a HeLa cell library into a baculovirus transfer vector, and protein was expressed in Sf21 insect cells. Protein was purified by a combination of anion-exchange, size-exclusion, and affinity-chromatography steps, and was approximately 90% pure as shown by SDS−PAGE and N-terminal sequencing. TACE was assayed using the same substrate used for MMP-1 and -2 in 10 mM Hepes, containing 0.0075% Brij35, pH 7.5. Inhibitor testing and calculation of K_i values were done as described for the MMPs.

Cellular TNFα Release Assay. Mononuclear cells (hPBMCs) were isolated from the blood of healthy donors by centrifugation over Ficoll. Cells were washed in PBS, counted, and cultured in RPMI/5% FCS at 37 °C and 5% CO_2 in 96-well plates. Final cell density was 10^5 cells/100 μL. Cells were incubated with an inhibitor (7 concentrations) for 30 min and then stimulated by LPS (5 μg/mL) and interferon-γ (100 units/mL) for 3 h. Plates were centrifuged, and TNFα concentrations in the supernatants were determined by ELISA.

Rat LPS Challenge Model. Conscious male Sprague-Dawley rats, kept under standard conditions, were orally dosed by gavage with aqueous solutions of compounds **1** and **3**−**5** at

Scheme 1[a]



[a]Conditions: (a) BnOH, NaOH, CH_2Cl_2, Bu_4NHSO_4, 48%; (b) 4-MeO-phenylacetic acid, DBU, CH_2Cl_2, 69%; (c) Li-HMDS, TMS-Cl, $TiCl_4$, THF, −78 → 20 °C, 99%; (d) (−)-1-(*S*)-phenethylamine, 31%;. (e) L-*tert*-leucine methylamide, EDCI, HOBT, NEt_3, DMF, 93%; (f) NMM-N-oxid, OsO_4, *tert*-butyl alcohol/water, 100%; (g) $NaIO_4$, acetone/water, 100%; (h) $NaClO_2$, 2-methyl-2-butene, *tert*-butyl alcohol/water, 46%; (i) BnO-NH_2, EDCI, HOBT, DMF, 46%; (j) H_2, Pd-$BaSO_4$, MeOH, 93%.

Table 1. Inhibition of TACE and TNFα Production in hPBMCs and MMPs by Succinate-Type Hydroxamate Inhibitors[a]

compound	TACE K_i (nM)	TNFα IC_{50} (nM)	MMP-1 K_i (nM)	MMP-2 K_i (nM)	MMP-3 K_i (nM)
1	6.3	1001	0.7	0.6	8.9
2	12.9	1606	0.6	0.3	9.8
3	4.9	928	3.5	4.8	13.2
4	3.8	269	2.0	0.3	15.2
5	0.6	48	1.1	0.5	0.9

[a] Inhibition constants, K_i, and IC_{50} values were determined as described in the Experimental Section.

doses between 0.1 and 30 mg/kg (4−7 rats per group; controls receiving vehicle only were included in the study). The rats were anesthetized 3 h later for the remaining experimental procedure. The carotid arteries (for blood sampling) and jugular veins (for LPS injections) were cannulated, and 1 h thereafter a single dose (500 μg/kg) of LPS was given. One hour later, arterial blood was taken, and plasma was prepared and, subsequently, was tested for TNFα concentrations (ELISA). TNFα inhibition of compound-treated groups was calculated as a percentage of controls treated with vehicle only. Additionally, an ED_{50} was determined for compound **5**.

Chemistry. Unless otherwise noted, materials were obtained from commercial suppliers and used without purification. Analytical thin-layer chromatography was performed with precoated glass-backed plates (Merck Silicagel 60 F_{254}). Compounds were purified either by flash chromatography using Merck Silicagel 60 (40−63 μm) or by preparative high-pressure liquid chromatography on RP-18 silica gel. NMR spectra were recorded on a Bruker MX-400 or DPX-400 spectrometer (400 MHz proton) in the indicated solvent. Chemical shifts are expressed as parts per million (ppm) downfield from tetramethylsilane, and *J* values are reported in hertz (Hz). Fast atom bombardment mass spectroscopy (Xe, 8 keV) on a VG70-SE mass spectrometer was used for all reported compounds. C,H,N analyses were carried out by Novartis Services AG with all substances biologically tested.

General Procedures for Compounds in Figure 1 and Table 1. The procedures used to synthesize the inhibitors in Table 1 are described in detail for compound **5**. Compounds **2**−**4** were synthesized in a similar fashion; however, the syntheses were carried through without separating the diastereomeric mixtures obtained after the Claisen−Ireland rearrangement. Separation of the diastereomers was done at the end of the synthesis by preparative HPLC on RP-18 silica using methanol/water as eluent. The stereochemistry of compounds **2**−**4** was assigned in analogy to compound **5** by the NMR shifts of the *tert*-butyl groups, the order of elution from the RP-HPLC, and their biological activity.

(4-Bromobut-2-enyloxymethyl)benzene (7). To a solution of *trans*-1,4-dibromobut-2-ene (50.00 g, 233.8 mmol), benzyl alcohol (26.6 mL, 257.2 mmol), and *tert*-butylammoniumhydrogensulfate (7.94 g, 23.4 mmol) in CH_2Cl_2 (200 mL) was added an aqueous solution of NaOH (113.7 mL, 2.1 mol). The mixture was stirred for 24 h at room temperature, diluted with water (400 mL), and extracted with Et_2O (2 × 500 mL). The combined organic extracts were dried over Na_2SO_4 and filtered, and the solvents were removed. The residue was purified by flash chromatography on silica using hexanes/EtOAc (9/1) as eluant to give **7** (27.04 g, 48%). ^{1}H-NMR ($CDCl_3$): 3.98 (d, 2H, J = 7.3 Hz), 4.05 (d, 2H, J = 4.4 Hz), 4.54 (s, 2H), 5.82−6.08 (m, 2H), 7.25−7.45 (m, 5H).

(4-Methoxyphenyl)acetic Acid 4-Benzyloxybut-2-enyl Ester (8). A solution of DBU (3.47 g, 22.8 mmol) in CH_2Cl_2 (10 mL) was added to a solution of **7** (5.0 g, 20.7 mmol) and (4-methoxyphenyl)acetic acid (3.44 g, 20.7 mmol) in CH_2Cl_2 (60 mL) within 15 min at room temperature. The reaction mixture was stirred for 24 h at room temperature; the CH_2Cl_2 was evaporated, and the residue was dissolved in EtOAc/water (1/1, 100 mL). The solution was extracted with EtOAc and dried over Na_2SO_4, and the solvent was removed. The residue was purified by flash chromatography on silica using hexanes/EtOAc (9/1) as eluant to give **8** (4.70 g, 69%). ^{1}H-NMR ($CDCl_3$): 3.58 (s, 2H), 3.79 (s, 3H), 4.03 (d, 2H, J = 4.1 Hz), 4.52 (s, 2H), 4.61 (d, 2H, J = 4.1 Hz), 5.80−5.90 (m, 2H), 6.85 (d, 2H, J = 8.7 Hz), 7.21 (d, 2H, J = 8.7 Hz), 7.30−7.40 (m, 5H). MS (DCI, CH_4) *m/e*: 326 (M^+).

3(*R*)-Benzyloxymethyl-2(*S*)-(4-methoxyphenyl)pent-4-enoic Acid (10). To a solution of hexamethyldisilazane (22.62 g, 140.0 mmol) in THF (400 mL) was added 1.6 M *n*BuLi (87.60 mL, 140.0 mmol) in hexane at 0 °C. After being stirred for 10 min, the reaction mixture was cooled to −78 °C, and tri-

methylchlorosilane (15.23 g, 140.0 mmol) was added, followed by a solution of **8** (30.50 g, 93.4 mmol) in THF (40 mL). After the mixture had been stirred for 1 h at −78 °C, 1 M $TiCl_4$ (1.89 mL, 1.9 mmol) in CH_2Cl_2 was added. The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature; the mixture was stirred for one additional hour, and the solvents were evaporated. The remaining residue was taken up in ether (500 mL) and extracted with 0.1 N NaOH (2 × 300 mL). The combined aqueous phases were acidified with 1 N HCl and extracted with ether (2 × 300 mL), and the combined organic layers were dried over Na_2SO_4. The solvent was evaporated to yield the racemic mixture **9** (30.4 g, 93.1 mmol, 99.6%) as a raw product. Crude product **9** was dissolved in ethanol (350 mL); (−)-1(*S*)-phenylethylamine (12.42 g, 102.4 mmol) was added, and the resulting suspension was heated to reflux while ethanol was added until a clear solution was obtained. The solution was allowed to cool to room temperature, and a crystalline product was isolated by filtration. It was recrystallized from ethanol (350 mL), and the isolated crystals were suspended in EtOAc (300 mL); 2 N HCl (200 mL) was added, and the resulting solution was extracted with EtOAc (2 × 300 mL). The combined organic layers were dried over Na_2SO_4, and the solvents were evaporated to yield acid **10** (9.3 g, 31%) as a single enantiomer. HPLC analysis showed a single peak t_R = 22.7 min (Chiralcel OD, *n*-hexanes/*i*-PrOH/TFA = 95/5/0.1) [t_R (anti-isomer) = 17.3/22.7 min/t_R (syn-isomer) = 15.4/20.1 min]. ^{1}H-NMR (DMSO): 2.98−3.05 (m, 1H), 3.41−3.55 (m, 2H), 3.59 (d, 1H, *J* = 10.2 Hz), 3.72 (s, 3H), 4.47 (s, 2H), 4.82−4.91 (m, 2H), 5.41−5.51 (m, 1H), 6.83 (d, 2H, *J* = 8.5 Hz), 7.18 (d, 2H, *J* = 8.5 Hz), 7.25−7.35 (m, 5H), 12.25 (s, 1H). MS (DCI, CH_4) *m/e*: 326 (M^+).

3(*R*)-Benzyloxymethyl-2(*S*)-(4-methoxyphenyl)pent-4-enoic Acid (2,2-Dimethyl-1(*S*)-methylcarbamoylpropyl)-amide (11). To a solution of **10** (3.00 g, 9.2 mmol) in DMF (25 mL) at 0 °C were added successively HOBT (1.55 g, 10.1 mmol), EDCI (1.76 g, 9.2 mmol), and triethylamine (2.79 g, 27.6 mmol). After 15 min, L-*tert*-leucine methylamide hydrochloride (2.0 g, 11.04 mmol) was added, and the mixture was stirred for 18 h at room temperature. The solvent was evaporated, and the residue was taken up in EtOAc (200 mL). The organic layer was extracted with 2 N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL) and dried over Na_2SO_4. The solvents were removed, and the residue was dried in vacuo to obtain **11** (4.2.g, 93%), which was used in the next step without further purification. ^{1}H-NMR (DMSO): 0.98 (s, 9H), 2.47 (s, 3H), 2.98−3.05 (m, 1H), 3.41−3.51 (m, 2H), 3.72 (s, 3H), 3.79 (d, 1H, *J* = 9.4 Hz), 4.16 (d, 1H, *J* = 9.4 Hz), 4.42 (s, 2H), 4.78−4.85 (m, 2H), 5.35−5.50 (m, 1H), 6.80 (d, 2H, *J* = 8.5 Hz), 7.24 (d, 2H, *J* = 8.5 Hz), 7.25−7.35 (m, 5H), 7.80−7.85 (m, 1H), 7.96 (d, 1H, *J* = 8.3 Hz). MS (FAB, Thio) *m/e*: 453 [(M + H)]$^+$.

2(*R*)-Benzyloxymethyl-*N*-(2,2-dimethyl-1(*S*)-methylcarbamoylpropyl)-3(*S*)-(4-methoxyphenyl)succinamic Acid (12). To a solution of **11** (4.05 g, 8.9 mmol) in acetone (60 mL) were added a solution of NMM-*N*-oxide (1.57 g, 11.6 mmol) in water (70 mL) and a 0.2 M solution of OsO_4 (1.34 mL, 0.27 mmol) in *tert*-butyl alcohol. The reaction mixture was stirred for 18 h at room temperature; aluminum oxide (1.6 g) and Na_2SO_3 (0.8 g) were added, and the mixture was filtered through silica gel. The filtrate was diluted with EtOAc (200 mL); the organic layer was washed with 2 N HCl, saturated $NaHCO_3$, and brine and dried over Na_2SO_4. The solvent was removed in vacuo, and the remaining bishydroxy compound (4.4 g, 100%) was dissolved in acetone (50 mL). To this solution was added $NaIO_4$ (2.08 g, 9.7 mmol) dissolved in water (50 mL) within 15 min. The reaction mixture was stirred for 4 h at room temperature, quenched with water, and extracted with EtOAc (2 × 200 mL). The organic layer was washed with brine and dried over Na_2SO_4, and the solvents were removed in vacuo. The remaining aldehyde (4.0 g, 100%) was dissolved in *tert*-butyl alcohol (70 mL) and 2-methyl-2-butene (20 mL). A solution of $NaClO_2$ (1.03 g, 11.4 mmol) and NaH_2PO_4 (1.37 g, 11.4 mmol) in water (20 mL) was added within 15 min. The reaction mixture was stirred for 18 h at room temperature, and the solvents were removed in vacuo. The remaining residue was taken up in ether (300 mL) and extracted with 2 N NaOH (2 × 200 mL). The aqueous layer was acidified with 2 N HCl and extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with brine and dried over Na_2SO_4, and the solvents were removed in vacuo. The remaining white solid was recrystallized from ether to obtain acid **12** (1.85 g, 46%) in the form of white crystals. ^{1}H-NMR (DMSO): 0.85 (s, 9H), 2.46 (d, 3H, *J* = 4.3 Hz), 3.25−3.32 (m, 1H), 3.45−3.50 (m, 1H), 3.59 (t, 1H, *J* = 9.4 Hz), 3.71 (s, 3H), 3.88 (d, 1H, *J* = 10.2 Hz), 4.13 (d, 1H, *J* = 10.2 Hz), 4.44 (s, 2H), 6.83 (d, 2H, *J* = 8.5 Hz), 7.28 (d, 2H, *J* = 8.5 Hz), 7.28−7.35 (m, 5H), 7.83 (q, 1H, *J* = 4.3 Hz), 8.13 (d, 1H, *J* = 8.7 Hz). MS (ESI) *m/e*: 493.3 [(M + Na)$^+$].

***N*^{1}-Benzyloxy-2(*R*)-benzyloxymethyl-*N*^{4}-(2,2-dimethyl-1(*S*)-methylcarbamoylpropyl)-3(*S*)-(4-methoxyphenyl)-succinamide (13).** To a solution of **12** (1.80 g, 3.8 mmol) in DMF (20 mL) at 0 °C were added successively HOBT (0.64 g, 4.2 mmol), EDCI (0.73 g, 3.8 mmol), and triethylamine (1.16 g, 11.5 mmol). After 15 min, *O*-benzylhydroxylamine hydrochloride (0.67 g, 4.2 mmol) was added, and the mixture was stirred for 18 h at room temperature. The solvent was evaporated, and the residue was taken up in EtOAc (200 mL). The organic layer was extracted with 2 N HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL) and dried over Na_2SO_4. The remaining solid was crystallized from EtOAc to obtain compound **13** (0.93 g, 42%) as a white solid. From the mother liquor, the solvents were removed in vacuo, and the remaining oil was purified by flash chromatography on silica gel using hexanes/EtOAc (3/7) as eluant to give an additional batch of **13** (0.53 g, 24%). ^{1}H-NMR (DMSO): 0.88 (s, 9H), 2.47 (d, 3H, *J* = 4.8 Hz), 3.08−3.17 (m, 1H), 3.35−3.41 (m, 1H), 3.62 (t, 1H, *J* = 9.6 Hz), 3.69 (s, 3H), 3.86 (d, 1H, *J* = 11.4 Hz), 4.12 (d, 1H, *J* = 11.4 Hz), 4.13 (d, 1H, *J* = 11.4 Hz), 4.31 (d, 1H, *J* = 11.4 Hz), 4.42 (s, 2H), 6.81 (d, 2H, *J* = 9.0 Hz), 7.12 (d, 2H, *J* = 9.0 Hz), 7.24−7.37 (m, 10H), 7.80 (q, 1H, *J* = 4.8 Hz), 8.12 (d, 1H, *J* = 9.0 Hz), 10.97 (s, 1H). MS (ESI) *m/e*: 576.4 [(M + H)]$^+$.

***N*^{4}-(2,2-Dimethyl-1(*S*)-methylcarbamoylpropyl)-*N*^{1}-hydroxy-2(*R*)-hydroxymethyl-3(*S*)-(4-methoxyphenyl)succinamide (5).** Compound **13** (0.90 g, 1.6 mmol) was hydrogenated in MeOH (80 mL) with Pd-$BaSO_4$ (0.80 g) under a hydrogen atmosphere. After the solution was stirred for 6 h, the catalyst was filtered off, and the solvent was removed in vacuo. The remaining solid was crystallized from water to yield the hydroxamate **5** (0.39 g, 63%) in the form of white crystals. ^{1}H-NMR (DMSO): 0.92 (s, 9H), 2.46 (d, 3H, *J* = 6.1 Hz), 2.87−2.94 (m, 1H), 3.43−3.51 (m, 1H), 3.52−3.60 (m, 1H), 3.71 (s, 3H), 3.84 (d, 1H, *J* = 12.7 Hz), 4.14 (d, 1H, *J* = 9.4 Hz), 4.66 (t, 1H, *J* = 4.3 Hz), 6.78 (d, 2H, *J* = 8.7 Hz), 7.27 (d, 2H, *J* = 8.7 Hz), 7.78 (q, 1H, *J* = 6.1 Hz), 8.03 (d, 1H, *J* = 9.5 Hz), 8.49 (s, 1H), 10.19 (s, 1H). MS (ESI) *m/e*: 394.2 [(M − H)]$^-$. Anal. ($C_{19}H_{29}N_3 \cdot 0.66H_2O$) C, H, N.

***N*^{4}-(2,2-Dimethyl-1(*S*)-methylcarbamoylpropyl)-*N*^{1}-hydroxy-2*(R)*-hydroxymethyl-3(*R*)-(isobutyl)succinamide (2).** ^{1}H-NMR (DMSO): 0.74 (d, 3H, *J* = 8.1 Hz), 0.79 (d, 3H, *J* = 8.1 Hz), 0.92 (s, 9H), 1.22−1.32 (m, 1H), 1.35−1.45 (m, 1H), 2.21−2.31 (m, 1H), 2.54 (d, 3H, *J* = 4.3 Hz), 2.56−2.63 (m, 1H), 3.22−3.32 (m, 1H), 3.48−3.55 (m, 1H), 4.15 (d, 1H, *J* = 10.2 Hz), 4.50 (t, 1H, *J* = 4.3 Hz), 7.72−7.78 (m, 2H), 8.71 (s, 1H), 10.35 (s, 1H). MS (FAB, Thio) *m/e*: 352 [(M + Li)]$^+$. Anal. ($C_{16}H_{33}N_3 \cdot H_2O$) C, H, N.

***N*^{4}-(2,2-Dimethyl-1(*S*)-methylcarbamoylpropyl)-*N*^{1}-hydroxy-2(*R*)-hydroxymethyl-3(*S*)-phenylsuccinamide (3).** ^{1}H-NMR (DMSO): 0.93 (s, 9H), 2.46 (d, 3H, *J* = 4.3 Hz), 2.92−3.01 (m, 1H), 3.43−3.51 (m, 1H), 3.52−3.60 (m, 1H), 3.93 (d, 1H, *J* = 10.2 Hz), 4.14 (d, 1H, *J* = 10.2 Hz), 4.68 (t, 1H, *J* = 4.3 Hz), 7.14−7.22 (m, 3H), 7.35−7.38 (m, 2H), 7.76 (q, 1H, *J* = 4.3 Hz), 8.09 (d, 1H, *J* = 9.4 Hz), 8.49 (s, 1H), 10.22 (s, 1H). MS (FAB,Thio) *m/e*: 366 [(M + H)]$^+$. Anal. ($C_{19}H_{29}N_3 \cdot 2.5H_2O$) C, H, N.

***N*^{4}-(2,2-Dimethyl-1(*S*)-methylcarbamoylpropyl)-*N*^{1}-hydroxy-2(*R*)-hydroxymethyl-3(*S*)-*p*-tolylsuccinamide (4).** ^{1}H-NMR (DMSO): 0.90 (s, 9H), 2.23 (s, 3H), 2.43 (d, 3H, *J* =

4.3 Hz), 2.89−2.95 (m, 1H), 3.43−3.51 (m, 1H), 3.52−3.60 (m, 1H), 3.83 (d, 1H, J = 10.2 Hz), 4.11 (d, 1H, J = 10.2 Hz), 4.60 (t, 1H, J = 4.3 Hz), 7.00 (d, 2H, J = 8.5 Hz), 7.22 (d, 2H, J = 8.5 Hz), 7.72 (q, 1H, J = 4.3 Hz), 7.98 (d, 1H, J = 9.4 Hz), 8.41 (s, 1H), 10.14 (s, 1H). MS (ESI) *m/e*: 378.1 $[(M - H)]^-$. Anal. ($C_{19}H_{29}N_3 \cdot 1.5H_2O$) C, H, N.

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Supporting Information Available: Methods used for the X-ray structure determination and data refinement for compound **5** as well as tables with bond lengths and angles. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Brennan, F. M.; Maini, R. N.; Feldmann, M. Role of Pro-Inflammatory Cytokines in Rheumatoid Arthritis. *Springer Semin. Immunopathol.* **1998**, *20*, 133−147.

(2) Feldmann, M.; Charles, P.; Taylor, P.; Maini, R. N. Biological Insights from Clinical Trials with Anti-TNF Therapy. *Springer Semin. Immunopathol.* **1998**, *20*, 211−228.

(3) Weinblatt, M. E.; Kremer, J. M.; Bankhurst, A. D.; Bulpitt, K. J.; Fleischmann, R. M.; Fox, R. I.; Jackson, C. G.; Lange, M.; Burge, D. J. A Trial of Etanercept, a Recombinant Tumor Necrosis Factor Receptor: Fc Fusion Protein, Patients with Rheumatoid Arthritis Receiving Methotrexate. *N. Engl. J. Med.* **1999**, *340*, 253−259.

(4) Clark, I. M.; Rowan, A. D.; Cawston, T. E. Matrix Metalloproteinase Inhibitors in the Treatment of Arthritis. *Curr. Opin. Anti-Inflammatory Immunomodulatory Invest. Drugs* **2000**, *2*, 16−25.

(5) Konttinen, Y. T.; Ainola, M.; Valleela, H.; Ma, J.; Mandelin, J.; Kinne, R. W.; Santavirta, S.; Sorsa, T.; López-Otin, C.; Takagi, M. Analysis of 16 Different Matrix Metalloproteinases (MMP-1 to MMP-16) in the Synovial Membrane: Different Profiles in Trauma and Rheumatoid Arthritis. *Ann. Rheum. Dis.* **1999**, *58*, 691−697.

(6) Black, R. A.; Rauch, C. T.; Kozlosky, C. J.; Peschon, J. J.; Slack, J. L.; Wolfson, M. F.; Castner, B. J.; Stocking, K. L.; Reddy, P.; Srinivasan, S.; Nelson, N.; Boiani, N.; Schooley, K. A.; Gerhart, M.; Davis, R.; Fitzner, J. N.; Johnson, R. S.; Paxton, R. J.; March, C. J.; Cerretti, D. P. A Metalloproteinase Disintegrin That Releases Tumor-Necrosis Factor-α from Cells. *Nature* **1997**, *385*, 729−733.

(7) Moss, M. L.; Jin, S.-L. C.; Milla, M. E.; Burkhart, W.; Carter, H. L.; Chen, W.-J.; Clay, W. C.; Didsbury, J. R.; Hassler, D.; Hoffman, C. R.; et al. Cloning of a Disintegrin Metalloproteinase That Processes Precursor Tumor-Necrosis Factor-α. *Nature* **1997**, *385*, 733−736.

(8) Maskos, K.; Fernandez-Catalan, C.; Huber, R.; Bourenkov, G. P.; Bartunik, H.; Ellestad, G. A.; Reddy, P.; Wolfson, M. F.; Rauch, C. T.; Castner, B. J.; Davis, R.; Clarke, H. R.; Petersen, M.; Fitzner, J. N.; Cerretti, D. P.; March, C. J.; Paxton, R. J.; Black, R. A.; Bode, W. Crystal Structure of the Catalytic Domain of Human Tumor Necrosis Factor-α-Converting Enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3408−3412.

(9) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. Design and Therapeutic Applications of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, *99*, 2735−2776.

(10) Xue, C.-B.; Voss, M. E.; Nelson, D. J.; Duan, J. J.-W.; Cherney, R. J.; Jacobson, I. C.; He, X.; Roderick, J.; Chen, L.; Corbett, R. L.; et al. Design, Synthesis, and Structure−Activity Relationships of Macrocyclic Hydroxamic Acids That Inhibit Tumor Necrosis Factor-α Release in Vitro and in Vivo. *J. Med. Chem.* **2001**, *44*, 2636−2660.

(11) Conway, J. G.; Andrews, R. C.; Beaudet, B.; Bickett, D. M.; Boncek, V.; Brodie, T. A.; Clark, R. L.; Crumrine, R. C.; Leenitzer, M. A.; McDougald, D. L.; et al. Inhibition of Tumor Necrosis Factor-α (TNF-α) Production and Arthritis in the Rat by GW3333, a Dual Inhibitor of TNF-α-Converting Enzyme and Matrix Metalloproteinases. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 900−908.

(12) Beckett, R. P.; Whittacker, M. Matrix Metalloproteinase Inhibitors 1998. *Expert Opin. Ther. Pat.* **1998**, *8*, 259−282.

(13) Barlaam, B.; Bird, T. G.; Lambert-van der Brempt, C.; Campbell, D.; Foster, S. J.; Maciewitz, R. New α-Substituted Succinate-Based Hydroxamic Acids as TNFα Convertase Inhibitors. *J. Med. Chem.* **1999**, *42*, 4890−4908.

(14) Nelson, F. C.; Zask, A. The Therapeutic Potential of Small Molecule TACE Inhibitors. *Expert Opin. Invest. Drugs* **1999**, *8*, 383−392.

(15) Hofmann-La, F.; Roche, A. G. Novel *N*-Hydroxamic Acid Hydrazide Inhibitors of TNF-α Synthesis. *Expert Opin. Ther. Pat.* **2000**, *10*, 1617−1621.

(16) Ireland, R. E.; Mueller, R. H.; Willard, A. K. The Ester Enolate Claisen Rearrangement. Stereochemical Control Through Stereoselective Enolate Formation. *J. Am. Chem. Soc.* **1976**, *98*, 2868−2877.

(17) Ireland, R. E.; Wilcox, C. S. A Stereoselective Method for the Generation of Aldol-Type Systems. *Tetrahedron Lett.* **1977**, 2839−2842.

(18) Stein, R. L.; Izquierdo-Martin, M. Thioester Hydrolysis by Matrix Metalloproteinases. *Arch. Biochem. Biophys.* **1994**, *308*, 274−277.

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