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A cassette-dosing approach for improvement of oral bioavailability of dual TACE/MMP inhibitors

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Abstract—The structural features contributing to the different pharmacokinetic properties of the TACE/MMP inhibitors TNF484 and Trocade™ were analyzed using an in vivo cassette-dosing approach in rats. This enabled us to identify a new lead compound with excellent pharmacokinetic properties, but weaker activity on the biological targets. Directed structural modifications maintained oral bioavailability and restored biological activity, leading to a novel compound almost equipotent to TNF484 in vivo, but with a more than tenfold higher oral bioavailability.

Matrix metalloproteases (MMPs) and the closely related family of a Disintegrin and Metalloprotease (ADAM) enzymes have attracted considerable interest as drug targets for the treatment of arthritis, periodontal disease, tumor metastasis, tumor growth, aneurysm, and atherosclerosis.[1–3] We recently described a series of β-aryl-succinic acid hydroxamates as highly potent dual inhibitors of TACE and matrix metalloproteinases.[4] Notably the key compound (2*S*,3*R*)-N^4-((*S*)-2,2-dimethyl-1-methylcarbamoyl-propyl)-N^1-hydroxy-2-hydroxymethyl-3-(4-methoxy-phenyl)-succinamide (TNF484) potently inhibited the release of the pro-inflammatory cytokine TNFα from cells as well as the LPS-induced systemic TNFα release in rats. This compound also showed in vivo activity in models of airway inflammation and pneumococcal meningitis.[5,6] Despite this impressive in vivo activity, its poor pharmacokinetic (PK) profile with an oral bioavailability (*F*) of approximately 3% in rats remained a major concern. Several other hydroxamate-type inhibitors, for example, Marimastat or Trocade™, underwent clinical trials for the indications of cancer and rheumatoid arthritis.[7,8] Trocade™ is structurally rather distinct from TNF484 (see Fig. 1) and has a reported absolute oral bioavailability of 26% in rats, as well as good tolerability and pharmacokinetics in arthritis patients.[9] Thus, we set out to determine structural features that had either beneficial or deleterious effects on the pharmacokinetic properties of Trocade™ and TNF484, respectively.

In an effort to establish a structure–PK relationship between these two compounds, we designed the three chimeras **1**, **2**, and **3** of TNF484 and Trocade™. The synthesis of Trocade™ was published by Broadhurst et al.[10] and our group has reported the process development of TNF484.[11] Accordingly, all our inhibitors were prepared following this methodology. The key step was the diastereoselective Lewis acid promoted Claisen–Ireland rearrangement[12] shown in the general Scheme 1.

The Claisen–Ireland rearrangement produced the racemic mixtures with erythro/threo ratios of typically 10:1 or better. The enantiomerically pure acids were obtained by crystallization with *S*-(−)-1-phenyl-ethylamine. After the coupling with appropriate amines, the olefins were subjected to ozonolysis followed by reductive workup with dimethylsufide. The crude aldehydes were used without further purification and oxidized to the carboxylic acids by sodium chlorite. Finally, the acids were activated with morpholinoethyl isocyanide (MEI) and 2-hydroxy-pyridine-*N*-oxide (HOPO) and then coupled with TMS-protected hydroxylamine. Simple hydrolysis with water gave the desired hydroxamic acids in good yields.

Assessing the pharmacokinetics in a timely fashion and with a high throughput[13] was of key importance for this project. In our hands, cassette-dosing[14] in conscious,

Keywords: TNFα; TACE; MMP; Metalloproteinase; Dual; Inhibitor; Pharmacokinetics; Cassette-dosing; Bioavailability; Oral; Clearance; Absorption; Rheumatoid arthritis; Trocade; TNF484; Claisen–Ireland rearrangement.
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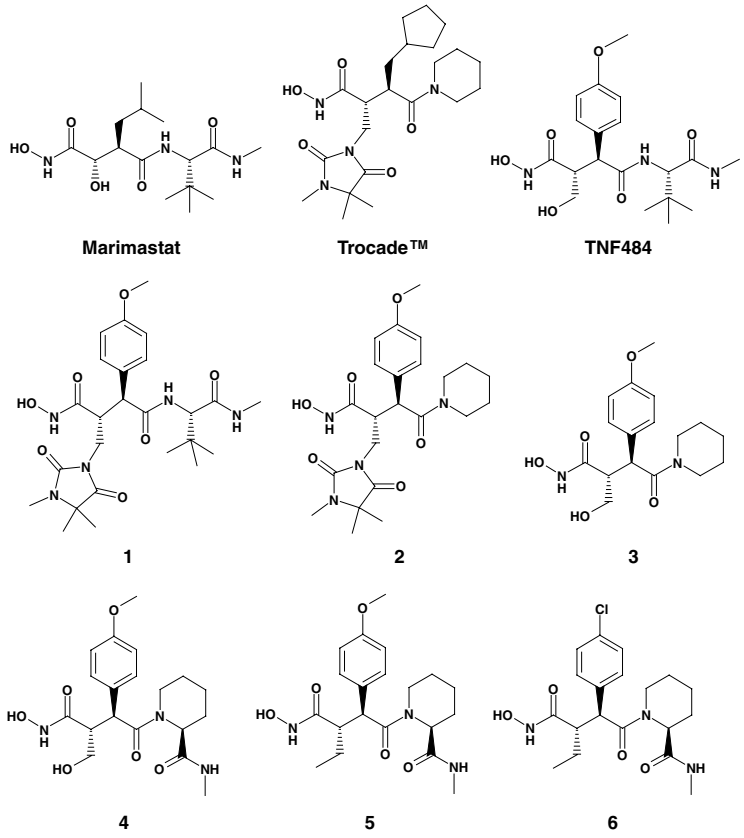
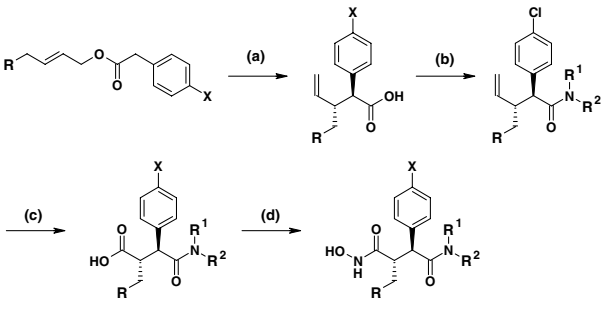


Figure 1. Chemical structures of TACE/MMP-inhibitors.

permanently cannulated rats[15] proved to be a very useful and reliable tool which guided our structural modifications toward inhibitors with considerably improved pharmacokinetic properties.

The results of our first cassette-dosing experiment are summarized in Table 1. For comparison, we have also included the pharmacokinetic profile of TNF484 when administered as single compound (last entry). The comparable outcome supports again the reliability of cassette-dosing within a series of similar compounds. We could confirm both, the low (3%) and the medium (32%) oral bioavailabilitiy (F) of TNF484 and Trocade™, respectively. Interestingly, we found that the higher bioavailability of Trocade™ did not lead to a much higher maximal blood concentration (C_{max}) which can be explained by the relatively high clearance (CL). Apart from the oral bioavailability, the most pronounced differences between Trocade™ and TNF484 were the low clearance and the small volume of distribution (V_{SS}) of the latter. This is typical for polar and hydrophilic compounds like TNF484 which have a high polar surface area (PSA) and a low $c\log P$. The combination of a low clearance with low oral bioavailability suggests that the latter is limited by a poor absorption rate. Replacing the hydroxymethyl group in TNF484 by the hydantoin residue in **1** further increased the polar surface area together with a slight increase in $c\log P$. This did not significantly affect the bioavailability, but it led to a further reduction of C_{max}, mainly due to a higher clearance. Replacement of the *tert*-butylglycine amide group by piperidine in **2** had a quite dramatic influence on the overall pharmacokinetic profile. Due to the higher lipophilicity, both clearance and volume of distribution were significantly higher but the absorption rate improved, which resulted in a better bioavailability of 13%. An even bigger effect was observed for **3** ($F = 36\%$), where the hydantoin residue was replaced with the original hydroxymethyl group present in TNF484. We attribute this substantial improvement mainly to a good absorption rate, combined with a slightly lower $c\log P$ and polar surface area, leading to a more than threefold reduction in clearance compared to Trocade™. This is also mirrored in the high C_{max} of 253 nM (dose-normalized to 1 mg/kg). To our



Scheme 1. Synthesis of succinic hydroxamates. Reagents: (a) LiHMDS, TMS-Cl, $TiCl_4$, THF; (b) HNR^1R^2, BOP-Cl, NEt_3, CH_2Cl_2; (c) i—ozone, MeOH; ii—Me_2S; iii—$NaClO_2$, NaH_2PO_4, *t*-BuOH, water; (d) i—MEI, HOPO, CH_2Cl_2; ii—$TMSONH_2$; iii—water.

Table 1. Pharmacokinetic parameters (means ± SEM) calculated from plasma levels after iv (1 mg/kg each) and po (3 mg/kg each) administration of a cocktail of five compounds to conscious rats ($n = 4$)

Compound	CL (mL/min/kg)	V_{SS} (L/kg)	C_{max} po[a] (nM)	F (%)	$c\log P$	PSA
TNF484	9.2 (±0.3)	0.37 (±0.02)	31.7 (±6.7)	2.8 (±0.4)	0.62	137.0
Trocade™	67.8 (±3.5)	2.31 (±0.19)	58.3 (±10.3)	31.6 (±6.8)	2.12	110.3
1	18.5 (±0.9)	0.48 (±0.04)	19.0 (±7.0)	3.2 (±0.8)	0.85	157.4
2	84.9 (±7.6)	2.45 (±0.28)	28.3 (±8.0)	13.0 (±2.0)	1.51	119.5
3	18.8 (±0.4)	1.08 (±0.03)	253.3 (±44.3)	35.9 (±5.2)	1.36	99.1
TNF484[b]	10.0 (±1.1)	0.34 (±0.03)	42.7 (±18.3)	3.2 (±0.7)	0.62	137.0

Results from an identically designed experiment where **TNF484** was administered as a single compound are shown for reasons of comparison.
[a] Mean from individual animals, dose-normalized to 1 mg/kg.
[b] Dosed as a single compound.

surprise, the anticipation that the hydroxymethyl group would be a metabolic weak point (e.g., oxidation, glucuronidation, sulfation, etc.) was not confirmed, and thus, we could identify **3** as a new lead compound with excellent pharmacokinetic properties, even better than Trocade™ (cf. Fig. 2).

Unfortunately, **3** was much less potent than TNF484 at inhibiting both the recombinant enzymes as well as the cellular TNFα release from human peripheral blood mononuclear cells (HPBMCs), as can be seen in Table 2. By comparison of the two pairs **3**/TNF484 and **1/2** it is obvious that the removal of the *tert*-butyl-glycine amide unit leads to improved pharmacokinetic properties, but at the same time is responsible for the loss of activity. Therefore, our further efforts were aimed at restoring the amide group to recover the activity, while maintaining the favorable PK properties.

First, we replaced the piperidine in **3** with pipecolic amides (cf. **4**). Despite a tenfold increase in potency on TACE, the cellular activity was still >10 μM. Introduction of an ethyl substituent instead of the hydroxymethyl group in **5** did not change TACE or MMP inhibition but improved the IC_{50} to 836 nM in the cellular TNFα release. Finally, we replaced the methoxy-phenyl substituent by chloro-phenyl and obtained with compound **6** a TACE inhibitor with a cellular IC_{50} of 316 nM. The PK properties of these compounds are summarized in Table 3.

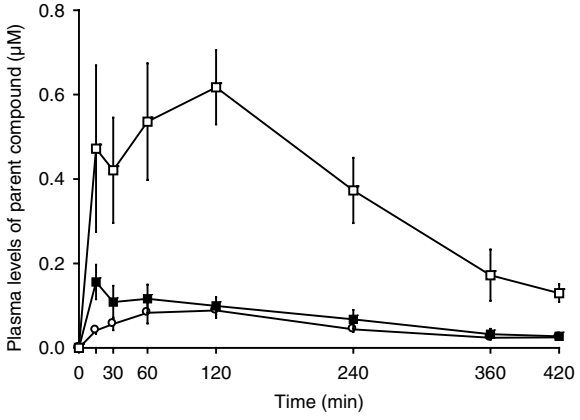


Figure 2. Plasma level time courses in rats ($n = 4$) for: ○, TNF484; ■, Trocade™; □, **3**. Shown are means ± SEM.

Table 2. In vitro potencies (measured in duplicate) for compounds

Compound	MMP-1[a] K_i (nM)	MMP-3[a] K_i (nM)	MMP-13[a] K_i (nM)	TACE[a] K_i (nM)	HPBMC[b] IC_{50} (nM)
TNF484	1	0.9	0.5	0.6	48
Trocade™	0.3	56	1	230	>10,000
1	0.7	12	3	57	484
2	83	187	19	321	>10,000
3	213	300	55	66	>10,000
4	23	n.d.	10	8	>10,000
5	25	11	7	8	836
6	0.7	5	2	5	316

[a] Determined by fluorimetric assay using recombinant enzymes.[16]
[b] Human peripheral blood mononuclear cells, stimulated with LPS/γ-interferon.[4]

Not unexpectedly, the quite polar inhibitor **4** still had an unfavorable pharmacokinetic profile, again indicating poor absorption. In contrast, the more lipophilic ethyl derivative **5** resembled more the profile of **3**, but this structural modification also increased the clearance which could be responsible for the lower bioavailability of only 20%.

A further increase in lipophilicity and a reduction of polar surface area, accompanied with the elimination of a possible metabolic weak point by replacing the *p*-methoxy group in **5** with a *p*-chloro substituent in **6**, proved to be highly beneficial. Compound **6** had a well-balanced pharmacokinetic profile, that is, moderate clearance, medium volume of distribution, and a good bioavailability of 65.7%. A further interesting aspect was the high maximal blood concentration of 452 nM (dose-normalized to 1 mg/kg). This result motivated us to perform a single compound administration experiment with compound **6** (last entry in Table 3). With the exception of the higher volume of distribution (indicating good tissue penetration) we could confirm the cassette-dosing study. A comparable oral bioavailability of 47% and a maximal blood concentration of 389 nM were found.

With this profile, we decided to test **6** in the LPS-induced systemic TNFα release model in rats (see Fig. 3).[17]

At the four doses tested (1, 3, 10, and 30 mg/kg) it inhibited TNFα release into plasma by 29%, 53%, 70%, and 94%, respectively. From these data we calculated an

Table 3. Pharmacokinetic parameters (means ± SEM) calculated from plasma levels after iv (1 mg/kg each) and po (3 mg/kg each) administration to conscious rats ($n = 4$)

Compound	CL (mL/min/kg)	V_{SS} (L/kg)	C_{max} po[a] (nM)	F (%)	$c\log P$	PSA
4[b]	23.2 (±1.0)	1.06 (±0.05)	38.1 (±11.0)	8.4 (±1.7)	0.75	128.2
5	68.0 (±2.6)	5.95 (±0.41)	48.0 (±15.0)	19.9 (±2.7)	1.29	108.0
6	43.3 (±2.3)	2.25 (±0.13)	452 (±67)	65.7 (±9.6)	2.08	98.7
6[b]	28.1 (±2.6)	16.67 (±2.37)	388.6 (±60.7)	47.1 (±8.3)	2.08	98.7

Inhibitors **5** and **6** were dosed as a cocktail of five compounds, whereas **4** was administered as single compound. Results from an identically designed experiment where **6** was administered as a single compound are shown for reasons of comparison.

[a] Mean from individual animals, dose-normalized to 1 mg/kg.

[b] Dosed as a single compound.

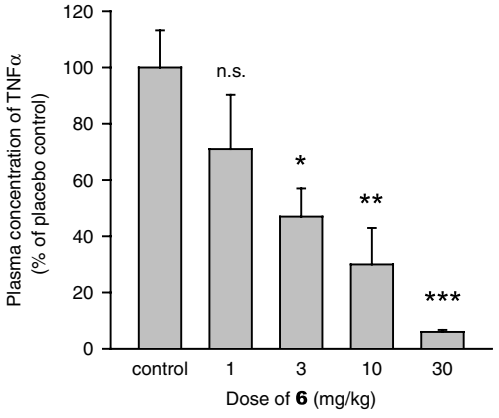


Figure 3. Release of TNFα into plasma of rats ($n = 4$–7) treated orally with four different doses of **6** compared to vehicle alone (=control). Shown are means ± SEM; n.s., not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

ED_{50} of 1.5 mg/kg, which is almost equipotent with TNF484 which had an ED_{50} of 1 mg/kg in the same model.[4]

Poor oral bioavailability and its associated high variability in exposure is a frequent cause for failure in clinical drug development. We have shown that cassette-dosing was a useful and reliable tool to assess the pharmacokinetics of a series of TACE/MMP-inhibitors in the rat. We successfully applied this technique to identify the key structural features that are responsible for the different pharmacokinetic properties of TNF484 and Trocade™. These findings proved to be instrumental in our efforts to improve the poor absolute oral bioavailability (3%) of the potent TACE/MMP-inhibitor TNF484. A new lead compound (**3**) could be identified with excellent pharmacokinetic properties, but significantly weaker potency in vitro. Further structural modifications guided by parallel screening for good in vitro potency and superior biopharmaceutical and pharmacokinetic properties resulted in the new inhibitor **6** which is equipotent to TNF484 in vivo, but has a more than tenfold higher absolute oral bioavailability (47%) in rats and thus a reduced variability in exposure. Considering that rat pharmacokinetics is often representative for the human situation, we anticipate that inhibitor **6** should have a smaller risk of clinical failure.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.02.042.

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15. *PK cassette-dosing in rats.* The experiment was performed in conscious, fed, permanently cannulated rats kept under standard conditions. For oral administration by gavage, a mixture of ethanol (3%) and cornoil was used as a vehicle (5 mL/kg), whereas for the intravenous route compounds were administered into the femoral vein as a solution in *N*-methyl-2-pyrrolidone (30%) in polyethylene glycol 200 (0.5 mL/kg). Plasma samples of approximately 70 μL were collected from the femoral artery for 24 h after iv administration and for 7 h after oral dosing. iv and po administration was in the same animals with a 48 h washout period between administrations. After acetonitrile precipitation of plasma samples (25 μL), parent drug concentrations in extracts were measured using a specific HPLC/MS method. Complete details of the procedure are given as supporting information.
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17. *Rat LPS challenge.* Conscious male Sprague–Dawley rats, kept under standard conditions, were orally dosed by gavage with aqueous solutions of **6** at doses between 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 one hour 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 the compound-treated groups was calculated as a percentage of controls treated with vehicle only. Additionally, an ED_{50} was determined.