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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,

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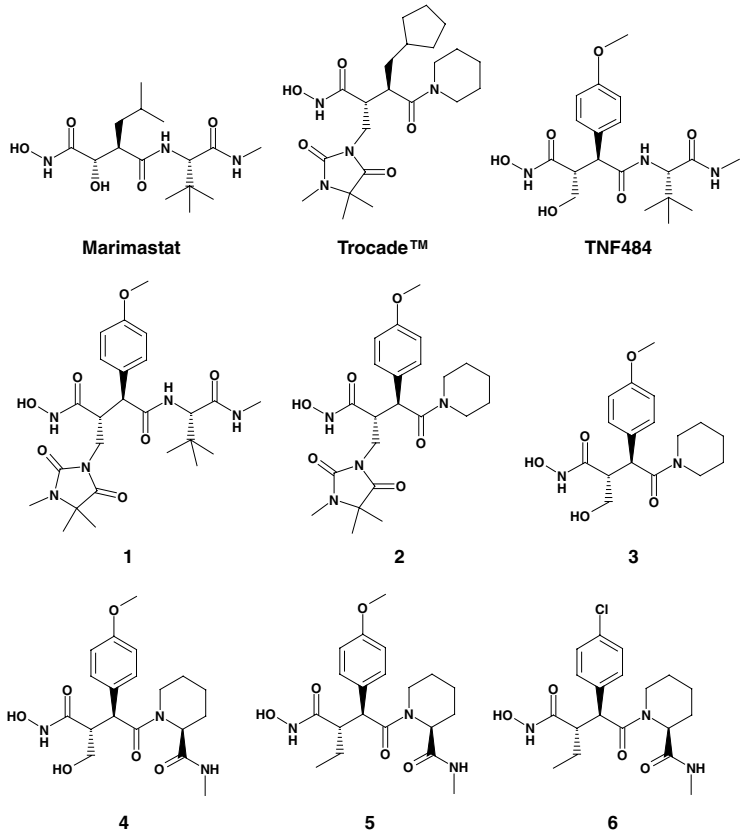
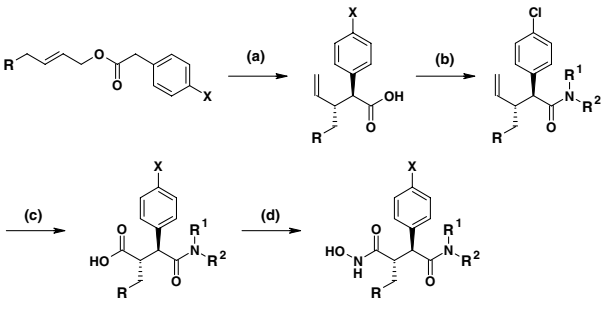



**Figure 1.** Chemical structures of TACE/MMP-inhibitors.

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.