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Reverse hydroxamate-based selective TACE inhibitors

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Abstract—Reverse hydroxamate-based selective TACE inhibitors are described. They have potent TACE inhibitory activities and excellent selectivities against MMP-1, 2, 3, 8, 9, 13, 14, and 17. One representative compound, **18** has demonstrated an excellent oral inhibitory activity of the lipopolysaccharide (LPS)-stimulated TNF-α production in rats.

Tumor necrosis factor α (TNF-α) is one of the major pro-inflammatory cytokines that is primarily or partly responsible for many kinds of inflammatory events within the living body.[1] The landmark success of currently marketed biologics targeting TNF-α, etanercept (Enbrel™), and infliximab (Remicade™), has clearly implicated TNF-α in rheumatoid arthritis,[2] and the other therapeutic indications are aggressively under exploration.[3] However, these two biologics are orally inactive and are inevitably administered parenterally. Furthermore, biologics commonly have a long half-life in the body and the above two biologics are not the exception. This may untimely cause infection incidents during the anti-TNF-α therapy, because TNF-α also plays an important role as a self-defense factor against pathogen.[4] Another concern is allergic reactions to the biologics and the neutralization antibody productions.[5] Based on these points of view, orally active anti-TNF-α small molecules with appropriate half-lives in the body are highly desirable.

TNF-α exists in two forms, the membrane-bound form (pro-TNF-α) and the soluble form (matured-TNF-α). Pro-TNF-α is processed into matured-TNF-α by a metalloproteinase. This metalloproteinase is called the TNF-α converting enzyme (TACE),[6] and TACE has been demonstrated to be the enzyme responsible for this process. More than 90% of matured-TNF-α is catalytically prepared by TACE and this fact implies that TACE may be one of the attractive targets in the anti-TNF-α therapy,[7] and as a matter of fact, there have been many reports on the TACE inhibitors.[8,9] The representative selective TACE inhibitors are shown in Figure 1.[8b,d,f]

TACE is structurally categorized into the ADAM (a disintegrin and a metalloprotease) family, but its catalytic site is quite similar to that of matrix metalloproteinases (MMPs).[10] MMPs are involved in the degradation and remodeling of connective tissues, and about 20 MMPs have been uncovered so far.[11] Each MMP should have distinctive or wide-ranging roles in the body, but the complete clarification of each MMP's functions leaves tremendous efforts still to be done. Some broad-spectrum and partially selective MMP inhibitors have been reported to cause musculoskeletal side effects in clinical trials.[12] Other experimental evidence that MMP-9 is essential for the liver regeneration[13] and that MMP-14-deficiency causes arthritis-like diseases[14] also supports that the inhibition of MMPs as foreboding difficulties to start a TACE inhibitor project for the treatment of RA. Accordingly, we tried to seek

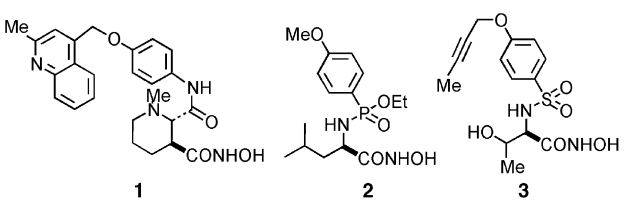

Figure 1. Selective TACE inhibitors.

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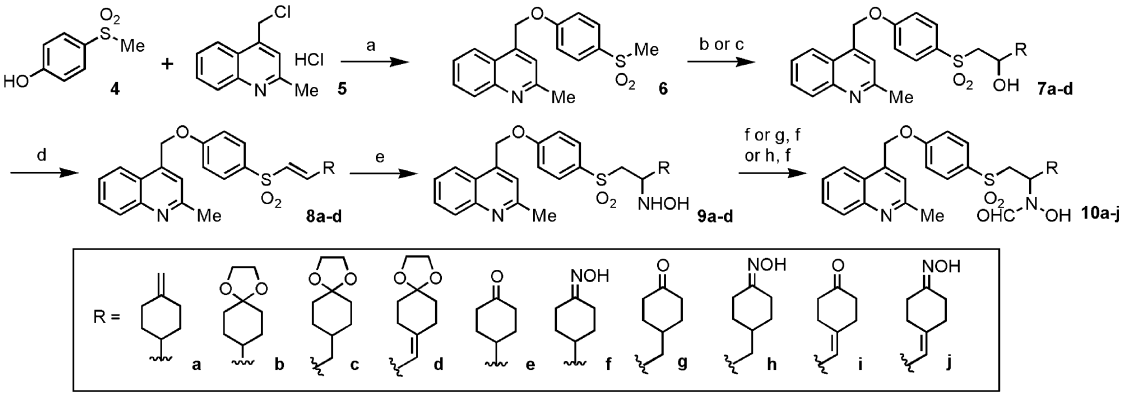
selective TACE inhibitors over MMPs to preclude any unexpected side effects caused by the inhibition of the MMPs.

Our TACE program started by exploring the SAR of a series of sulfonamide hydroxamate derivatives, and at the same time, by probing their pharmacokinetics, since the oral bioavailability and metabolic rate of a drug candidate often become a major concern in the late development stage. During this pilot study,[15] we observed that many of the prepared sulfonamide hydroxamate derivatives have a strong tendency toward hydrolysis of the hydroxamate moiety, eventually producing highly toxic hydroxylamine in the rodent blood plasma. Another drawback was the oral bioavailability. The accumulation of the SAR and the pharmacokinetics of the in-house TACE inhibiting compounds directed us toward the reverse hydroxamate (RH) derivatives[16] with which, we postulated, the aforementioned problems would be overcome. In this letter, we would like to describe the syntheses and biological profiles for a series of RH derivatives.

RHs **10a**–**j** were prepared as shown in Scheme 1. The coupling of phenol **4** and 4-chloromethyl-2-methylquinoline **5** under basic conditions gave the common intermediate **6**. After the deprotonation of **6** with lithium diisopropylamide, Julia coupling with a variety of aldehydes[17] gave rise to the corresponding alcohols **7c**–**d**. In the case of using esters[17] as an electrophile, the co-existence of stoichiometric lithium hexamethyldisilazide resulted in the corresponding β-ketosulfones with much better yields, which were successively reduced to alcohols **7a**–**b** in a one-pot procedure. Dehydration of alcohols **7a**–**d** afforded the α,β-unsaturated sulfones **8a**–**d**. The 1,4-addition of hydroxylamine to compounds **8a**–**d** afforded the hydroxylamine adducts **9a**–**d**, which were finally converted into the RH derivatives **10a**–**d** by the treatment of acetic formic mixed anhydride. The deprotection of the acetal group of **9b**–**d** afforded the free ketones, which were converted into the RH derivatives **10e**,**g**, and **10i**, respectively, in a similar fashion. The treatment of **9b**–**d** with hydroxylamine under acidic conditions provided the corresponding oximes, which were then converted into **10f**,**h**, and **10j**, respectively.

The inhibitory activities of compounds **10a**–**c**,**e**–**j** against TACE and MMPs (MMP-1, 2, 3, 8, 9, 13, 14, 17) are summarized in Table 1. Those against MMP-1, 8, 14 were very weak (>100,000 nM) and were omitted from the table. Compounds **10** exhibited excellent inhibitory activities if compared to the structurally similar

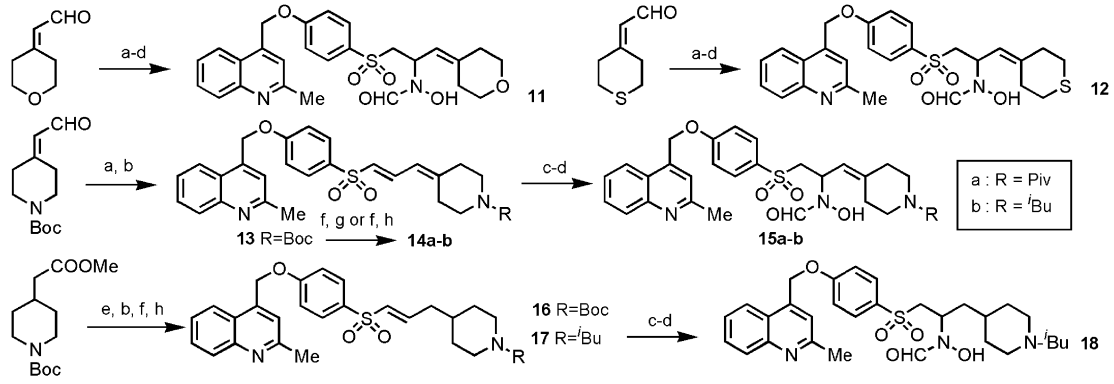


Scheme 1. Reagents and conditions: (a) 4 M NaOHaq, EtOH; (b) LDA, aldehyde; (c) LDA, LHMDS, ester, AcOH, $NaBH_4$, MeOH; (d) MsCl, Et_3N; (e) 50% NH_2OHaq, THF; (f) HCOOH, Ac_2O, THF or pyridine, MeOH; (g) 2 M HClaq; (h) 2 M HClaq, 50% NH_2OHaq.

Table 1. Inhibitory activities of reverse hydroxamates **10a**–**c**,**e**–**j**, **11**, **12**, **15a**,**b**, and **18** against TACE and MMPs[a]

Compound	TACE[b]	MMP-2[b]	MMP-3[b]	MMP-9[b]	MMP-13[b]	MMP-17[b]
10a	45	33,000	6000	>100,000	7300	2000
10b	15	7000	280	40,000	3300	220
10c	60	6000	1200	12,000	5000	1100
10e	20	30,000	2000	28,000	7000	600
10f	9.0	22,000	1600	20,000	7000	500
10g	4.5	15,000	1300	53,000	3300	350
10h	3.0	8000	550	40,000	4000	330
10i	3.5	41,000	6300	50,000	22,000	2500
10j	2.0	30,000	5000	45,000	15,000	3000
11	2.2	60,000	5500	53,000	25,000	3000
12	3.0	55,000	10,000	31,000	25,000	4000
15a	3.0	7000	1500	28,000	7000	550
15b	6.0	>100,000	10,000	>100,000	40,000	4000
18	12	5000	1500	90,000	4000	350

[a] Inhibitory activities against MMP-1, 8, 14 were weak (IC_{50} > 100,000 nM) and were not shown at the table.
[b] IC_{50}, nM.



Scheme 2. Reagents and conditions: (a) **6**, LDA, aldehyde; (b) MsCl, Et_3N; (c) 50% NH_2OHaq, THF; (d) HCOOH, Ac_2O, THF or pyridine, MeOH; (e) **6**, LDA, LHMDS, ester, AcOH, $NaBH_4$, MeOH; (f) 2 M HCl; (g) PivCl, Et_3N; (h) ^{i}PrCHO, $NaBH(OAc)_3$, AcOH.

hydroxamate derivatives,[15] though the RH group is known to have a less potent zinc binding capacity than that of the hydroxamate group.[18] This fact suggests that the character of the RH group is favorable for the binding to the catalytic site of TACE. The TACE inhibitory activities among **10a**,**e**, and **10f** showed that the capacities of the hydrogen bond acceptor and/or hydrogen bond receptor might contribute for the enhancement of the inhibitory activity. The one methylene carbon insertion between the cyclohexyl ring and the carbon directly attached to the RH group increased the TACE inhibitory activity shown by the comparison between **10e**,**f**, and **10g**,**h**, respectively. Another interesting and delightful finding was that the existence of the double bond definitely increased the TACE selectivity over MMPs. The RH group itself also slightly increased the TACE selectivity.

Transformation of the cyclohexyl group into a heterocyclic group was carried out to examine whether there was a favorable improvement in the activity and the selectivity, and at the same time, in order to compare the difference in the pharmacokinetics properties between the cyclohexyl compounds and the heterocyclic compounds. The heterocyclic compounds were synthesized in the same fashion as that of the cyclohexyl compounds (Scheme 2).[17] Compound **13** was subjected to deprotection of the Boc group, followed by the treatment of pivaloyl chloride and Et_3N, to afford the pivalamide **14a**. On the other hand, compounds **13** and **16** were transformed into compounds **14b** and **17**, respectively, by deprotection of the Boc group and the following reductive amination. Compounds **14a**–**b** and **17** were converted into the corresponding RHs **15a**–**b** and **18**, respectively.

The inhibitory activities of compounds **11**, **12**, **15a**–**b**, **18** are summarized in Table 1. The inhibitory characteristics of each individual compound possessing the heterocyclic group were in much the same with those of cyclohexyl compounds. The double bond effect remained intact and the comparison between **15b** and **18** clearly demonstrated that this double bond should have special abilities for the TACE selectivity, though it is still unclear whether the characteristics of the double bond itself or the three-dimensional shape of the six-membered ring restricted by the double bond accounts for this difference.

The series of RH derivatives we have explained in this letter had fairly good to excellent pharmacokinetics. The human plasma protein binding ratios of the cyclohexyl compounds **10** were just about 95%. Compound **10a** had an excellent oral bioavailability, 53% in rodent and was stable in the rodent blood plasma. Heterocyclic compounds **15b** and **18** had much better human plasma protein binding ratios (81% and 74%, respectively) and both of them have proved satisfactory in terms of solubility.

The in vivo TACE inhibitory activities of compound **18** was measured by the inhibition of the LPS-stimulated TNF-α production in rats. The compound, orally administered at 30 mg/kg 4 h prior to the LPS injection, provided the 92% reduction of the blood TNF-α levels,[19] and the blood levels of **18** was well above the IC_{50} against TACE.

In summary, we have created a series of RH derivatives with excellent TACE inhibitory activities and selectivities against the tested MMPs. The SAR of our compounds suggested that the cyclohexyl moiety has a strong influence on both the TACE inhibitory activities and the selectivities over MMPs. The pharmacokinetics of our compounds, especially with the heterocyclic substituent, was satisfactory as a drug candidate and the stability in the rodent blood plasma was effectively improved by changing the zinc binding group from the hydroxamate moiety to the RH moiety. Since all of the compounds explained above are racemics, the optically pure forms would have the preferable characteristics.

Acknowledgements

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19. Compound **18** was administered orally (30 mg/kg: 5% DMSO, 10 mM HCl, 0.5% methylcellulose) (feeding) 4 h prior to the intravenous injection of LPS (1 mg/kg). After 90 min, the blood was collected from the tail vein and the plasma was prepared. The plasma concentrations of TNF-α were measured by ELISA (DIACLONE). The TNF-α concentrations from an $n = 4$ rats group receiving the test compound were compared to those from a control group receiving vehicle alone ($n = 6$–7).