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Synthesis and Biological Activity of Piperazine-Based Dual MMP-13 and TNF-α Converting Enzyme Inhibitors

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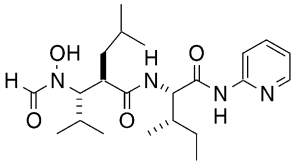
Abstract—A series of novel MMP-13 and TNF-α converting enzyme inhibitors based on piperazine 2-hydroxamic acid scaffolds are described. The TACE, MMP-1 and MMP-13 activity of these inhibitors as well as the effect of substitution of the piperazine nitrogen and the P-1′ benzyloxy tailpiece is discussed. Moderate in vivo activity is observed with several members of this group.

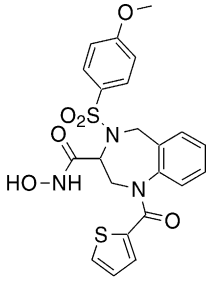
The pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is produced by monocytes/macrophages and other cell types. In a variety of diseases including rheumatoid arthritis (RA) and Crohn's disease (CD), the overproduction of TNF-α plays a key role in inducing the recruitment of inflammatory cells and in stimulating the production of other mediators of pain and cartilage breakdown.[1] Support for the therapeutic utility of molecules that inhibit the pro-inflammatory effects of TNF-α includes results of recent human clinical trials for two biologics, etanercept, a soluble TNF-α receptor-Fc dimer (Immunex/Amgen), and infliximab, an anti-TNFα antibody (Centocor/Johnson and Johnson), in RA and CD.[2] Both of these biologics are currently marketed for the treatment of rheumatoid arthritis and both have been shown to decrease signs and symptoms and to reduce joint destruction in RA patients.

TNF-α is synthesized as a 26 kDa membrane protein that is shed from the cell following a proteolytic cleavage carried out by a 90 kDa membrane-bound zinc metalloprotease, TNF-α Converting Enzyme (TACE).[3–5] Once cleaved, sol-TNF-α is liberated from the cell and it is this soluble form of TNF-α that is thought to be responsible for much of the inflammation associated with RA and other inflammatory diseases.



GW3333

TACE IC_{50}= 40 nM
MMP-13 IC_{50}=7 nM
MMP-1 IC_{50}=19 nM



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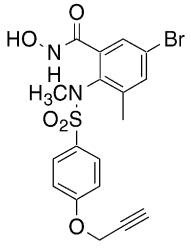
TACE IC_{50}= 13 nM
MMP-13 IC_{50}=1 nM
MMP-1 IC_{50}=20 nM



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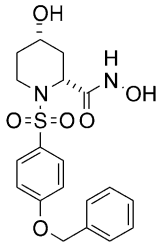
TACE IC_{50}= 11 nM
MMP-1 IC_{50}=52 nM
MMP-13 IC_{50}=113 nM



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rTACE IC_{50}= 9 nM
MMP-1 IC_{50}=8000 nM

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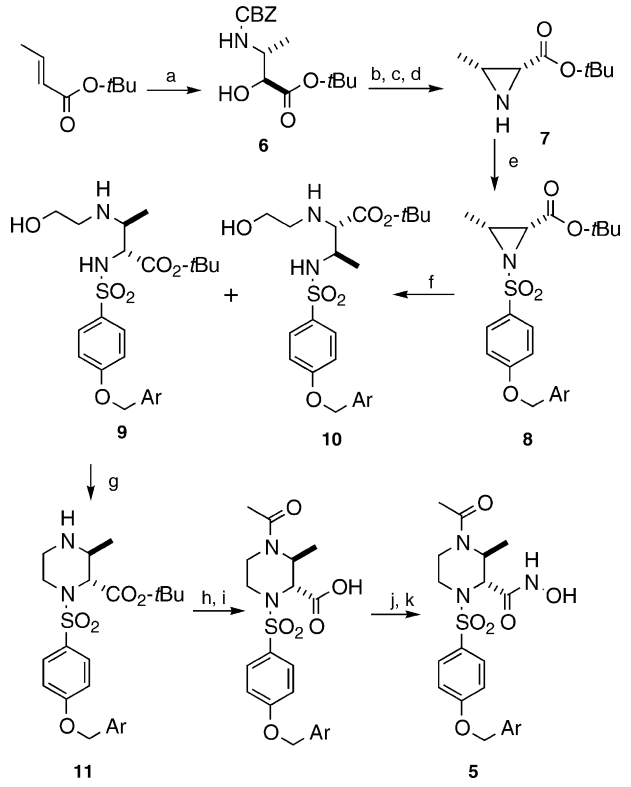
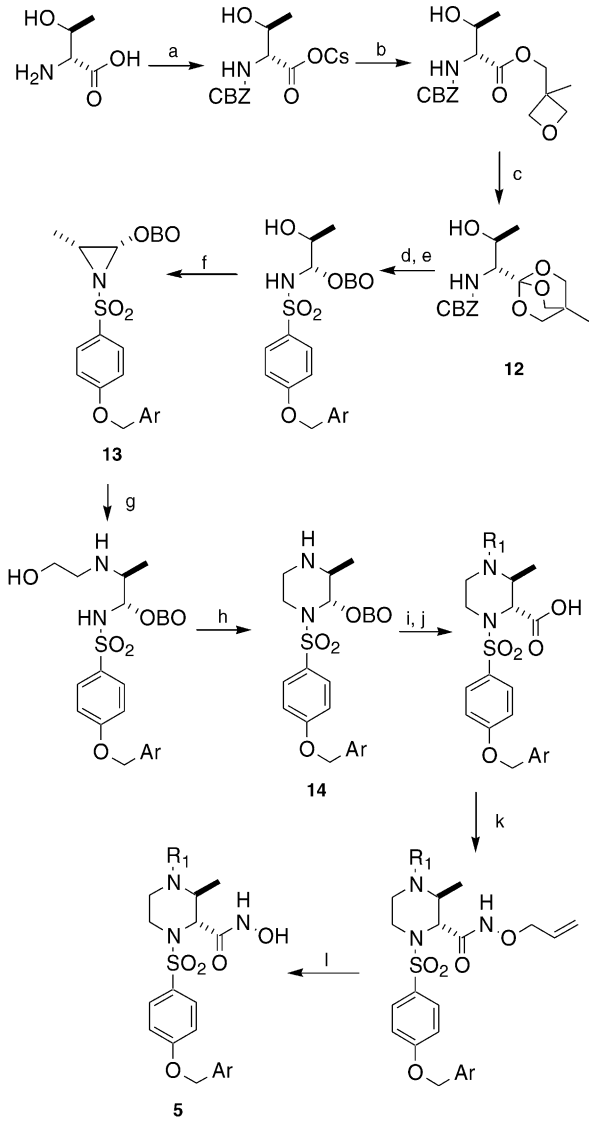


Scheme 1. (a) $(DHQD)_2PHAL$, $K_2OsO_2(OH)_4$, CBZNHCl, *t*-BuOCl, 60%; (b) methanesulfonyl chloride, Et_3N,CH_2Cl_2, 78%; (c) KO*t*-Bu, 30–40%; (d) H_2, Pd/C, EtOH, 86%; (e) $ArSO_2Cl$, Et_3N, DMF, 96%; (f) 2-aminoethanol, CH_2Cl_2, 47%; (g) Ph_3P, DEAD, 90%; (h) acetyl chloride, Et_3N, DMF, 100%; (i) TFA, CH_2Cl_2, 100%; (j) *O*-allylhydroxylamine, EDC, HOBt, DIPEA, 50–68%; (k) $Pd(Ph_3P)_4$, HCO_2H/Et_3N, 21–55%.

Therefore we,[6] and others,[7–9] have had an interest in molecules that have the potential to inhibit TACE in vivo, thereby reducing the circulating levels of sol-TNF-α. Our group at Pfizer has also had a long-standing interest in the use of matrix metalloprotease inhibitors (MMP inhibitors) for the treatment of arthritis and a variety of other disease states.[10] More specifically, we were interested in molecules that are potent MMP-13 inhibitors as MMP-13 has been reported to be expressed in human osteoarthritic cartilage and recombinant MMP-13 has been shown to efficiently cleave type II collagen in vitro.[11] We were also seeking compounds with diminished activity against MMP-1 hypothesizing that this might eliminate some of the side effects reported to be associated with broad spectrum MMP inhibition. Based on the above information we began a program to discover small molecule inhibitors of TACE and MMP-13 that were devoid of MMP-1 activity with the idea that these molecules should be useful for the treatment of a variety of disorders. Several examples of dual MMP/TACE inhibitors have been reported in the literature recently, including GW3333,[9] the benzodiazepines[12] and the anthranilates[7,8] shown below. In addition to the structures shown, we recently disclosed the discovery of a series of pipecolic acid-based TACE inhibitors[6] and Procter and Gamble has recently reported on a series of broad-spectrum piperazine-based MMP inhibitors.[13] Following up on these efforts, we now describe the activity of a novel series of piperazine based hydroxamic acids that are dual TACE/MMP-13 inhibitors with minimal MMP-1 inhibition. We also report in vivo efficacy in an acute model of inflammation and pharmacokinetic data in rats for several of these inhibitors.

The preparation of the piperazines **5** was completed in one of two ways. Scheme 1 describes the initial route to these compounds, which utilizes an asymmetric aminohydroxylation[14] of *t*-butyl crotonate to form the aminoalcohol **6**. The alcohol of **6** is then converted to the mesylate upon treatment with methansulfonyl chloride. Subsequent cyclization in the presence of potassium *t*-butoxide provides the aziridine in moderate



Scheme 2. (a) Benzyl chloroformate, then cesium carbonate, 100%; (b) 3-bromomethyl-3-methyloxetane, DMF, 78%; (c) BF_3–Et_2O, 36%; (d) H_2, Pd/C, EtOH, 100%; (e) $ArSO_2Cl$, Et_3N, DMF, 87%; (f) Ph_3P, DEAD; (g) 2-aminoethanol; (h) Ph_3P, DEAD; (i) R^1-X, R-NCO, R-C(O)Cl or R-SO_2Cl; (j) trifluoroacetic acid then Cs_2CO_3, 50–70%, (k) *O*-allylhydroxylamine, EDC, HOBt, DIPEA, 40–68%; (l) $Pd(Ph_3P)_4$, HCO_2H:Et_3N, 22–55%.

Table 1. Biological data for piperazine analogues, **5**

Compd	R^1	Ar	rTACE (IC_{50}, nM)	Whole blood (IC_{50}, μM)	MMP-13 (IC_{50}, nM)	MMP-1 (IC_{50}, nM)
5a	H	Ph	25	18	11	12,000
5b	H	2-MePh	19	2	19	12,000
5c	$H_3CC(O)$	2-MePh	6	1	3	1600
5d	$H_3CC(O)$	2-EtPh	7	0.6	40	6700
5e	$H_3CC(O)$	3,5-FPh	6	1	20	3800
5f	$H_3CC(O)$	4-FPh	6	2	3	1700
5g	$H_3CC(O)$	2-Me, 5-FPh	7	1	7	1900
5h	$H_3CC(O)$	2-CF_3Ph	11	2	127	10,000
5i	$H_3CC(O)$	4-Isoquinoline	ND	0.4	98	6400
5j	$H_3CC(O)$	4-Quinoline	ND	0.3	17	830
5k	$H_3CC(O)$	3-(2-Me)-pyridyl	5	0.8	8	3400
5l	$MeSO_2$	2-MePh	6[a]	1	4	1300
5m	4-$MePhSO_2$	2-MePh	32[a]	26	ND	ND
5n	Me	2-MePh	31[a]	33	ND	ND
5o	$PhCH_2$	2-MePh	68[a]	33	ND	17,000
5p	PhNHC(O)	2-MePh	26[a]	12	ND	2200
5q	$H_3COC(O)$	2-MePh	14[a]	2	4	1500
5r	*i*-PrNHC(O)	2-MePh	34[b]	3	4	1400
5s	EtOC(O)	2-MePh	28[b]	3	6	1500

[a]As assayed using a rTACE assay.
[b]As assayed using a partially purified TACE membrane preparation.

yield. Hydrogenation to give aziridine **7** is followed by formation of the sulfonamide **8**. Reaction with 2-aminoethanol provides a mixture of aminoalcohols **9** and **10**. Chromatographic separation followed by the treatment of **9** with PPh_3/DEAD gives the piperazine **11** which can be converted to the corresponding piperazine hydroxamic acid by treatment with an appropriate electrophile, removal of the *t*-butyl ester, formation of the allyl hydroxamate and palladium mediated removal of the allyl moiety. This route has a number of issues. First, the sequence is rather lengthy, second, aziridine **7** is volatile and therefore difficult to handle and third, the ring opening reaction of **8** proceeds with little selectivity for the desired product **9**. In order to address these issues, a second route to these molecules was developed.

In the modified route (Scheme 2) D-threonine is converted to the *N*-CBZ protected 4-methyl-2,6,7-trioxabicyclo[2.2.2]oct-1-yl ester (OBO ester) in a four-step procedure that provides **12** in moderate yield. Conversion of **12** to the aziridine **13** is straightforward and provides a very sterically hindered aziridine to perform the ring opening reaction with 2-aminoethanol. Regioselective ring opening followed by reaction with PPh_3/DEAD gives the desired piperazine **14** in 65% overall yield. Acylation of the piperazine nitrogen is followed by removal of the OBO ester (TFA then Cs_2CO_3). The synthesis of analogues **5** is then completed as was described in Scheme 1. All of the compounds in Table 1 were prepared using slight modifications of these two procedures.

Table 2. In vivo data for piperazine analogues, **5**

Compd	Rat TNF ED_{50} (mg/kg)	Rat Cl_p (mL/min/kg)	V_{dss} (L/kg)	iv half-life (h)
5c	17	54	0.6	0.27
5b	39% inhib@30 mg/kg	83	1.1	0.24
5l	55% inhib@30 mg/kg	98	2.1	0.21

In vitro and human whole-blood data for a number of piperazines **5** is shown in Table 1. Variations of the substituents on the benzyl tailpiece and the piperazine nitrogen were examined. Most of the compounds shown are potent TACE inhibitors (<50 nM).[15] With the exception of the 2-trifluoromethylphenyl and 4-isoquinoline compounds **5i–j**, all of the compounds shown also have good MMP-13 activity.[16] The human whole-blood activity of these analogues varies considerably. This variation is likely caused by a number of factors, including protein binding and permeability. However, the ortho substituted aryl groups such as **5c–d** and **5k** and the 4-isoquinoline and 4-quinoline derivatives **5i–j** are clearly the most potent compounds in whole blood, with several compounds less than 500 nM. As expected from previous modeling[6] the benzyl tailpiece on these analogues is too large to fit in the smaller P-1′ pocket of MMP-1 and therefore none of the compounds shown has significant MMP-1 activity.

Compounds **5b**, **5c** and **5l** were examined in an acute rat LPS-induced model of systemic TNF-α production.[17] The iv pharmacokinetic parameters for these compounds were also determined in a rat.[18] The data are shown in Table 2. Of the compounds shown **5c** is the most potent in vivo (ED_{50} = 17 mg/kg). The moderate in vivo activity observed for these compounds is very likely due the their short half-life in rat which appears to be primarily due to high clearance.

In summary, these piperazine-based hydroxamic acids represent a new sub-type of potent dual TACE/MMP-13 inhibitors with potent TNF activity in human whole blood. The moderate in vivo activity of these analogues is likely due to high clearance in the rat. Further optimization of the in vivo properties of these and related TACE/MMP-13 inhibitors will be reported in due course.

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16. Compounds were assayed versus MMP-13 using a quenched fluorescent peptide substrate assay as described in Bickett, D. M.; Green, M. D.; Berman, J.; Dezube, M.; Howe, A. S.; Brown, P. J.; Roth, J. T.; McGeehan, G. M. *Anal. Biochem*. **1993**, *212*, 58.
17. In this model, the compounds are administered orally as a suspension of 0.5% methylcellulose. LPS injection leads to a systemic response, accompanied by release of TNF-α into the plasma. At 150 min post dose, plasma samples are acquired and cytokine levels are determined by ELISA.
18. The compound is administered iv; at a dose of 5 mg/kg as a solution in glycerol-formal.