Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1387–1390

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Biological Activity of Selective Pipecolic Acid-Based TNF-α Converting Enzyme (TACE) Inhibitors

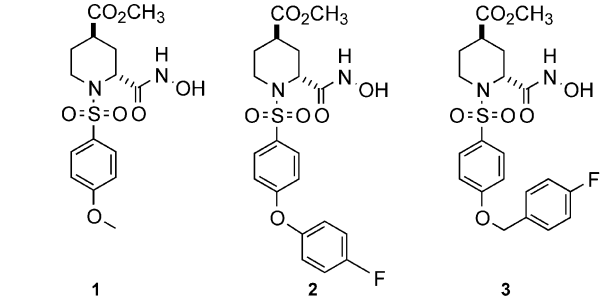
Michael A. Letavic,* Matt Z. Axt, John T. Barberia, Thomas J. Carty, Dennis E. Danley, Kieran F. Geoghegan, Nadia S. Halim, Lise R. Hoth, Ajith V. Kamath, Ellen R. Laird, Lori L. Lopresti-Morrow, Kim F. McClure, Peter G. Mitchell, Vijayalakshmi Natarajan, Mark C. Noe, Jayvardhan Pandit, Lisa Reeves, Gayle K. Schulte, Sheri L. Snow, Francis J. Sweeney, Douglas H. Tan and Chul H. Yu

Pfizer Global Research and Development, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA

Received 1 January 2002; accepted 5 March 2002

Abstract—A series of novel, selective TNF-α converting enzyme inhibitors based on 4-hydroxy and 5-hydroxy pipecolate hydroxamic acid scaffolds is described. The potency and selectivity of TACE inhibition is dramatically influenced by the nature of the sulfonamide group which interacts with the S1′ site of the enzyme. Substituted 4-benzyloxybenzenesulfonamides exhibit excellent TACE potency with >100× selectivity over inhibition of matrix metalloprotease-1 (MMP-1). Alkyl substituents on the *ortho* position of the benzyl ether moiety give the most potent inhibition of TNF-α release in LPS-treated human whole blood.

Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine produced by monocytes/macrophages and other cell types. In diseases such as rheumatoid arthritis (RA) and Crohn's disease (CD), dysregulated cellular release of TNF-α participates in the recruitment of inflammatory cells and stimulation of the production of other mediators of pain and cartilage breakdown (e.g., prostaglandins and MMPs, respectively).[1] In addition, data from human clinical trials have supported the therapeutic utility of two anti-TNF biologics, etanercept, a soluble TNF-α receptor-Fc dimer (Immunex), and infliximab, an anti-TNFα antibody (Centocor), in RA and CD.[2] TNF-α is synthesized as a membrane-anchored 26 kDa precursor. Proteolysis of the Ala76-Val77 peptide bond leads to the mature cytokine being shed from the cell as a homotrimer of the 17 kDa C-terminal fragment. This processing step is performed by a 85 kDa membrane-anchored zinc metalloprotease, TNF-α converting enzyme (TACE).[3–5] As one of several strategies to intervene in the production of TNF-α, we sought a small molecule inhibitor of TACE to control the level of extracellular TNF-α. The following describes our efforts using a series of pipecolates containing a hydroxamate moiety to form a bidentate ligand to the catalytic zinc atom of the enzyme, thus inhibiting the TACE-mediated release of soluble TNF-α from the cell.



1
TACE IC_{50} = 9600 nM
MMP-1 IC_{50} = 19 nM

2
TACE IC_{50} = 120 nM
MMP-1 IC_{50} = 8.5 nM

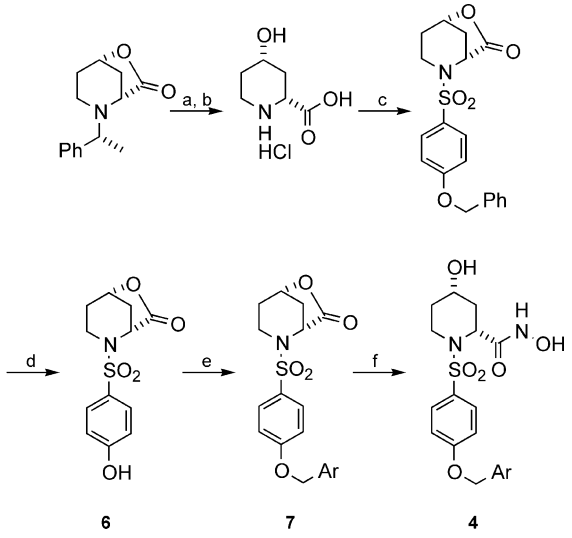
3
rTACE IC_{50} = 5 nM
MMP-1 IC_{50} = 1600 nM

During a search for novel MMP inhibitors we discovered a series of endocyclic hydroxamic acids that are potent TACE inhibitors with good selectivity over MMP-1. Thus, starting from hydroxamic acids such as **1** that have only weak TACE activity (IC_{50} = 9600 nM)[6] and significant MMP-1 activity (19 nM), we prepared compound **2** where the methoxy tailpiece in **1** is replaced with a 4-fluorophenoxy substituent. Compound **2** shows

*Corresponding author. Tel.: +1-860-441-1803; fax: +1-860-715-2467; e-mail: michael_a_letavic@groton.pfizer.com

0960-894X/02/$ - see front matter
PII: S0960-894X(02)00183-X

moderate TACE activity ($IC_{50} = 120$ nM)[6] while retaining potent MMP-1 activity. Further improvements in TACE activity were observed upon installation of a 4-fluorobenzyloxy substituent (**3**, $IC_{50} = 5$ nM against rTACE). The addition of the benzyloxy substituent in **3** also led to the erosion of MMP-1 activity ($IC_{50} =$ 1600 nM). In order to optimize the in vitro and in vivo activity of these endocyclic hydroxamic acids we have explored the biological properties of the related 4-hydroxy and 5-hydroxy pipecolic acid based hydroxamic acids **4** and **5** (Schemes 1 and 2). The hydroxy substituents on the pipecolic acid moiety were installed to lower the overall logP and potentially improve the physicochemical properties of the molecule (the addition of the hydroxy group in **4b** lowers the mlogP of the molecule from 2.1 to 1.3). We installed various benzyloxy tailpieces to these novel endocyclic hydroxamic acids in order to assess the TACE and MMP-1 activity in relation to changes at this site of the inhibitor.

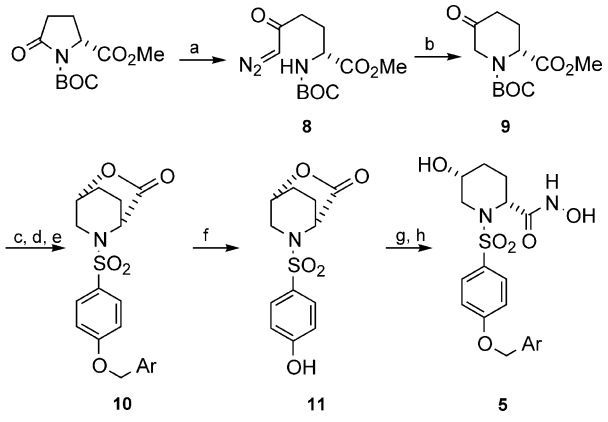


Scheme 1. (a) H_2, Pd/C, MeOH; (b) 6 N HCl; (c) 4-(benzyloxy)-phenylsulfonyl chloride, triethylamine, DMF, 80%; (d) H_2, Pd/C, MeOH, 84%; (e) Cs_2CO_3, $ArCH_2X$, DMF, 58–99%; (f) NH_2OH, MeOH, 60 °C, 36–94%.

For the purpose of preparing a wide range of benzyloxy analogues of the 4-hydroxypipecolate scaffold **4**, phenol **6** was prepared from 4-hydroxypiperidine-2-carboxylic acid[7] by treating the amine with 4-(benzyloxy)phenylsulfonyl chloride in the presence of triethylamine followed by hydrogenation of the resulting benzyloxyether. Alkylation of the phenol with the appropriate benzyl halide in the presence of cesium carbonate gave the required benzyloxylactones **7**, which were subsequently converted to hydroxamic acids **4** upon treatment with hydroxylamine in methanol.

The preparation of the corresponding 5-hydroxy pipecolates **5** is shown in Scheme 2. The intermediate diazoketone **8** was generated on treating an ether solution of the methyl ester of *N*-Boc-pyroglutamic acid with the anion of TMS-diazomethane at −100 °C.[8] Rhodium catalyzed cyclization[9] of **8** afforded the 5-oxopipecolic acid derivative **9**, which was stereoselectively reduced with methanolic sodium borohydride. Concomitant removal of the Boc group and lactonization of the hydroxy ester, followed by sulfonylation with 4-benzyloxyphenylsulfonyl chloride afforded the pipecolate lactone **10**. Hydrogenolysis of the benzyl ether of **10** using palladium adsorbed on activated charcoal afforded the 5-hydroxy pipecolate scaffold phenol **11**, which could be transformed into the corresponding pipecolic hydroxamic acids **5** as described in Scheme 1.

Figure 1 depicts the X-ray co-crystal structure of **5k** bound to TACE.[10] The binding mode of the hydroxamic acid and sulfonamide moieties are typical for this class of metalloprotease inhibitors;[11] the hydroxamic acid chelates to the catalytic zinc, and a sulfonamide oxygen accepts a hydrogen-bond from the main chain.



Scheme 2. (a) Trimethylsilyldiazomethane, *n*-butyllithium, Et_2O, −100 °C, 96%; (b) $Rh_2(OAc)_4$, benzene, reflux, 97%; (c) $NaBH_4$, MeOH, 96%; (d) 6 M HCl, reflux, 100%; (e) 4-benzyloxyphenylsulfonyl chloride, Et_3N, DMF, 57%; (f) H_2, Pd/C, MeOH, 99%; (g) K_2CO_3, $ArCH_2X$, DMF; (h) NH_2OH, MeOH, reflux, 30–47%.

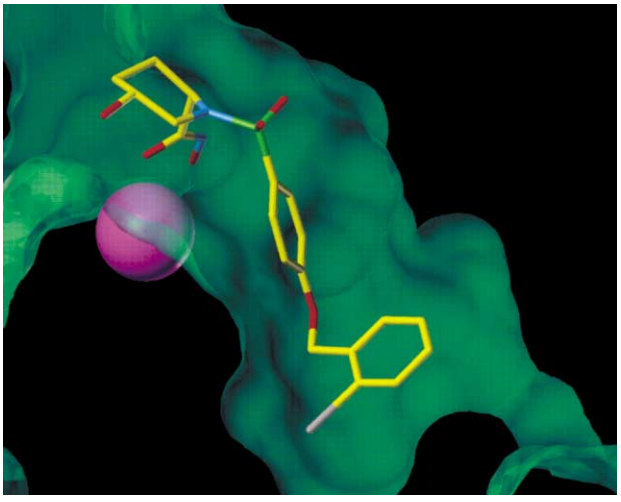


Figure 1. X-ray crystal structure of **5k** complexed with rTACE. The local solvent-accessible surface is rendered in greenblue, with the catalytic zinc portrayed as a violet CPK sphere. Atom colors are as follows: carbon, yellow; nitrogen, blue; oxygen, red; sulfur, green; iodine, gray.

Table 1. Biological data for 4-hydroxy analogues, **4**

Compd	Ar	rTACE IC_{50} (nM)	Whole blood IC_{50} (μM)	MMP-1 IC_{50} (nM)
4a	Phenyl	9	ND[a]	8000
4b	4-Fluorophenyl	14	12	ND
4c	3-Fluorophenyl	21	9	16,000
4d	2-Fluorophenyl	67	>30	12,000
4e	4-Chlorophenyl	7	58	6300
4f	3-Chlorophenyl	10	>30	6500
4g	2-Chlorophenyl	36	40	29,000
4h	4-Methylphenyl	20	>30	30,000
4i	3-Methylphenyl	18	>30	9000
4j	2-Methylphenyl	6	12	5000
4k	3-Methoxyphenyl	27	>30	24,000
4l	2-Methoxyphenyl	14	26	2200
4m	2-Trifluoromethylphenyl	16	16	ND
4n	4-Cyanophenyl	25	77	30,000
4o	3-Cyanophenyl	15	33	23,000
4p	2-Phenylphenyl	67	>33	ND

[a]ND, not determined.

Table 2. Biological data for 5-hydroxy analogues, **5**

Compd	Ar	rTACE IC_{50} (nM)	Whole blood IC_{50} (μM)	MMP-1 IC_{50} (nM)
5a	Phenyl	7	14	30,000
5b	4-Trifluoromethyl	10	28	3700
5c	3-Trifluoromethylphenyl	15	>30	5800
5d	2-Trifluoromethylphenyl	5	4	15,000
5e	3-Cyanophenyl	49	63	ND[a]
5f	2-Cyanophenyl	39	42	15,000
5g	2-Methylphenyl	8	3	3000
5h	2-Ethylphenyl	10	1	ND
5i	2-Isopropylphenyl	11	1	ND
5j	1-Naphthyl	16	2	ND
5k	2-Iodophenyl	7	1	ND

[a]ND, not determined.

Despite the elongated benzyloxy P1′ group, the binding conformation permits the two aryl rings to adopt positions similar to that reported for phenoxyphenyl substituents.[11] The *ortho* iodo substituent protrudes into a sub-pocket of S1′ that consists of the side chains of Val440 and Asn447 and the mainchain atoms of Ile438, Tyr436, Val434, and Tyr433. Comparison with the first published X-ray structure of TACE (PDB accession 1BKC)[12] reveals that accommodation of the iodo substituent requires significant conformational movement on the part of the enzyme. In the present structure Val440 is displaced by 2.4 Å relative to the previously reported structure and the backbone of Gly442 inverts and moves 5.7 Å to accommodate the inhibitor. The selectivity over MMP-1 that is observed with these inhibitors is attributed to the large P1′ substituent that is not accommodated by the shallow S1′ pocket of MMP-1.[11]

The hydroxamic acids in Tables 1 and 2 were assayed for TACE activity using a recombinant TACE assay[13] and a human whole blood assay measuring TNF-α.[14] Many of the analogues were also screened against MMP-1.[15] No significant MMP-1 activity was observed with any member of this series.

An examination of the rTACE activity within this series indicates that an *ortho* substituent on the benzyloxy ring is tolerated when the substituent is an electron-donating group. Hence the unsubstituted benzyl analogue **4a** has approximately the same potency as **4j** (2-Me) and **5a** is roughly equipotent with **5g–i** (2-Me, 2-Et and 2-*i*-Pr). However, when the substituent on the aryl ring is large (i.e., 2-phenyl, **4p**) there is significant loss of rTACE activity. Halogens in the *ortho* position (2-chloro and 2-fluoro) result in a loss of rTACE activity, but these substituents are tolerated at the *meta* and *para* positions of the ring.

Substitution at the *ortho* position of the terminal aryl ring has a more dramatic effect on the human whole blood potency. The inactivity of the 2-fluoro and 2-chloro analogues **4d** and **4g** in human whole blood might have been expected from the loss of rTACE activity observed with these compounds. However, significantly improved potency in human whole blood was observed, particularly in the 5-hydroxy series **5**, when *ortho* alkyl groups were introduced. Thus compound **5a** is 5- to 14-fold less potent than the substituted analogues **5g–i** in human whole blood in spite of the fact that the rTACE IC_{50}'s for the compounds are nearly identical. Also, the 5-hydroxypipecolates **5** are generally more potent in human whole blood than the 4-hydroxypipecolates **4**. For example, **4j** has an IC_{50} of 12 μM in human whole blood, but the corresponding 5-hydroxypipecolate **5g** has an IC_{50} of 3 μM. The 2-ethyl and 2-isopropyl analogues **5h** and **5i** are even more potent

(IC_{50}'s = 1 μM). The reasons for this are not entirely clear. Presumably the shift in activity observed in the human whole blood assay as compared to the rTACE assay is largely due to cell permeability requirements in combination with plasma protein binding issues.

The excellent human whole blood potency observed with these substituted pipecolic acid derivatives has prompted us to investigate the in vivo properties of the more potent members of this series. The results of these studies will be reported in due course.

References and Notes

1. Beutler, B. A. *J. Rheumatol.* **1999**, *26*, 16.
2. Feldmann, M.; Maini, R. N. *Annu. Rev. Immunol.* **2001**, *19*, 163.
3. 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. *Nature* **1997**, *385*, 729.
4. 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.; Kost, T. A.; Lambert, M. H.; Leesnitzer, M. A.; McCauley, P.; McGeehan, G.; Mitchell, J.; Moyer, M.; Pahel, G.; Rocque, W.; Overton, L. K.; Schoenen, F.; Seaton, T.; Su, J.-L.; Warner, J.; Willard, D.; Becherer, J. D. *Nature* **1997**, *385*, 733.
5. Subsequent to the completion of the research described in this manuscript a patent was issued to the Immunex Corporation covering the preparation of recombinant TACE. (US Patent Number 5,830,742; *Chem. Abstr.* **1997**, *126*, 154445).
6. As assayed using a membrane fraction prepared from MonoMac6 cells obtained from Dr. H. W. L. Ziegler-Hietbrock (Institute of Immunology, Munich, Germany) according to a published procedure (Maeda T.; Balakrishnan K.; Mehdi, S. Q. *Biochim. Biophys. Acta* **1983**, *731*, 115). TACE activity was assessed using a synthetic substrate peptide (ref 4, Moss, M. L.; Becherer, J.; Milla, M.; Pahel, G.; Lambert, M.; Andrews, R.; Frye, S.; Haffner, C.; Cowan, D.; Maloney, P.; Dixon, E.; Jansen, M.; Vitek, M.; Mitchell, J.; Leesnitzer, T.; Warner, J.; Conway, J.; Bickett, D.; Bird, M.; Priest, R.; Reinhard, J.; Lin, P. In *Metalloproteinases as Targets for Anti-Inflammatory Drugs*; Bradshaw, D., Nixon, J. S., Bottomley, K., Eds.; Birkhauser: Basel, 1999; p 187).
7. 4-Hydroxypiperidine-2-carboxylic acid was obtained in optically pure form using the procedures described in Gillard, J.; Abraham, A.; Anderson, P. C.; Beaulieu, P. L.; Bogri, T.; Bousquet, Y.; Grenier, L.; Guse, I.; Lavallee, P. *J. Org. Chem.* **1996**, *61*, 2226.
8. Coutts, I. G. C.; Saint, R. E. *Tetrahedron Lett.* **1998**, *39*, 3243.
9. Ko, K.-Y.; Lee, K.-I.; Kim, W.-J. *Tetrahedron Lett.* **1992**, *33*, 6651.
10. TACE catalytic domain (a 29,267 Da fragment encompassing residues 215–474 of human TACE with N264A and N452A mutations) was crystallized as a complex with **5k**. Protein at 1 mg/mL (35 μM) in 25 mM HEPES, 100 mM NaCl, pH 7.5 was treated with **5k** and then concentrated to 22 mg/mL. Crystals which diffracted to 2.0 Å grew from hanging drops set up with equal volumes of protein and well solution equilibrated over 26–28% PEG 4K, 0.1 M Tris, 0.2 M $MgCl_2$ (1–2 weeks). The crystals are orthorhombic (space group $P2_12_12_1$, a = 77.8, b = 81.7, c = 85.9 Å), and contain two copies of the protein-inhibitor complex in the crystallographic asymmetric unit. Data to 2.0 Å resolution were collected from a cryo-cooled crystal on a RAXIS IIC detector mounted on a Rigaku RU200 X-ray generator. The structure was solved by molecular replacement, using the coordinates of unliganded TACE (ref 11, PDB accession code 1BKC), as a starting search model in the program AmoRe (Navaza, J. *Acta Crystallogr.* **1994**, *A50*, 157). Difference Fourier maps calculated after initial refinement of the molecular replacement solution showed unambiguous electron density at the active site for the bound inhibitor molecule. The overall protein structure is essentially superimposable on the coordinates of 1BKC, with an r.m.s. difference of 0.63 Å for 255 C-α atoms.
11. Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campbell, J. A.; Walker, K. A. M.; Martin, R.; Van Wart, H.; Browner, M. F. *Nature Struct. Biol.* **1999**, *6*, 217.
12. 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. G.; Peterson, M.; Fitzner, J. N.; Cerretti, D. P.; March, C. J.; Paxton, R. J.; Black, R. A.; Bode, W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3408.
13. Enzyme reaction was comprised of 20 mM Hepes buffer (pH, 7.5), 20 μM $ZnCl_2$, 10 μM fluorescent quenched substrate, test compound, and rTACE enzyme. The reaction was started by the addition of substrate and initial rates of cleavage were monitored by increase in fluorescence at 530 nm (excitation at 409 nm) over 30 min. The specificity of the enzyme cleavage at the amide bond between alanine and valine was verified by HPLC and mass spectrometry. The fluorogenic substrate was prepared as described in Geoghegan, K. F. *Bioconjugate Chem.* **1996**, *7*, 385. Recombinant TACE catalytic domain was expressed with its prodomain in baculoviral infected insect (SF9) cells. Active enzyme was secreted into the extracellular medium and partially purified by anion-exchange chromatography on Q Sepharose Fast Flow (Pharmacia Biotech).
14. Heparinized human whole blood was stimulated by *Escherichia coli* LPS in the presence of test agent in volume of 0.25 mL for 4 h at 37 °C, after which plasma was recovered and TNF-α measured by ELISA (R&D Systems).
15. Compounds were assayed versus MMP-1 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.