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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2970–2973
Bioorganic & Medicinal Chemistry Letters

Non-hydroxamate 5-phenylpyrimidine-2,4,6-trione derivatives as selective inhibitors of tumor necrosis factor-α converting enzyme

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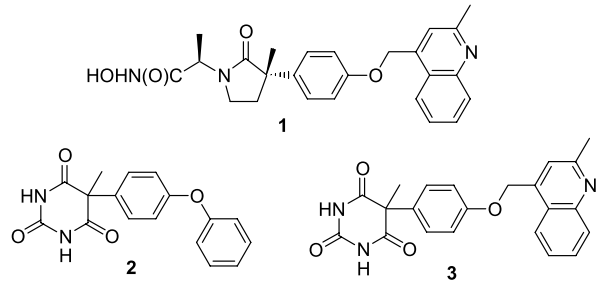
Received 25 March 2005; revised 21 April 2005; accepted 22 April 2005

Abstract—New inhibitors of tumor necrosis factor-α converting enzyme (TACE) were discovered with a pyrimidine-2,4,6-trione in place of the commonly used hydroxamic acid. These non-hydroxamate TACE inhibitors were developed by incorporating a 4-(2-methyl-4-quinolinylmethoxy)phenyl group, an optimized TACE selective P1′ group. Several leads were identified with IC_{50} values around 100 nM in a porcine TACE assay and selective over MMP-1, -2, -9, -13, and aggrecanase.

Tumor necrosis factor-α (TNFα), a cytokine produced mainly by activated macrophages and monocytes, is a major mediator of inflammatory and immune responses.[1] Clinically, TNF antibodies (infliximab and adalimumab) and soluble receptor (etanercept) have enjoyed great success in treatment of diseases, including Crohn's disease, rheumatoid arthritis, juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and psoriasis. As a result, many approaches to intersect the TNFα signaling pathway with orally active small molecules are being pursued.[2] One such approach is through the inhibition of TNFα converting enzyme (TACE), the enzyme responsible for the release of TNFα from cells.[3]

TACE is a membrane-bound zinc endopeptidase. Due to structure similarities between the active sites of TACE and matrix metalloproteinases (MMPs), early TACE inhibitors were derived from MMP inhibitors and consequently suffered from broad spectrum activity against the MMP family. Recently, inhibitors with selectivity for TACE against MMPs have been reported.[4] One example from our laboratories is lactam **1**.[5] The 4-(2-methylquinolin-4-ylmethoxy)phenyl group in **1** was designed and optimized to bind to the S1′ specificity pocket of TACE, and was shown to be the critical determinant for TACE selectivity. The generality of this group was demonstrated through the conversion of several series of potent MMP inhibitors to inhibitors that are selective for TACE against MMPs.[6]



To the best of our knowledge, TACE inhibitors in the literature predominantly rely on hydroxamic acid and related 'reverse hydroxamic acid' (*N*-hydroxyformamide) ligands to achieve the desired potency.[4] Because hydroxamic acids are often poorly absorbed in vivo and carry potential metabolic liabilities (hydrolysis and glucoronidation), there remains considerable interest in the discovery of non-hydroxamate TACE inhibitors. In recent years, there has been great progress to replace the hydroxamic acid group in MMP inhibitors.[7] One such example is a series of novel pyrimidine-2,4,6-trione based MMP inhibitors reported by scientists at Roche.[8] A crystal structure of a pyrimidinetrione analogue **2** in

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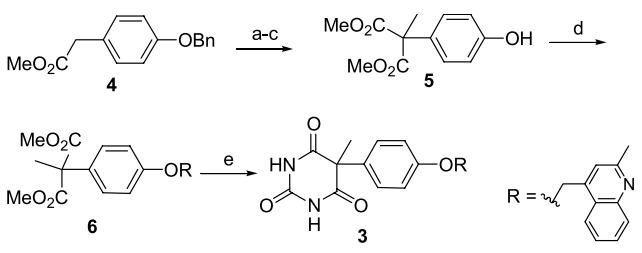
doi:10.1016/j.bmcl.2005.04.039

MMP-3 revealed that the pyrimidine-2,4,6-trione not only served as a ligand for the catalytic zinc but also was involved in several hydrogen-bonding interactions in the vicinity of the active site.[8c] Additionally, the 4-phenoxyphenyl group was projected into the S1′ pocket of MMP-3. We were intrigued by this binding mode observation and proceeded to explore the utility of the pyrimidinetrione as an alternative Zn ligand to the hydroxamate TACE inhibitors. Based on the structure of **2**/MMP-3 complex, we proposed that the 4-phenoxyphenyl group could be replaced with 4-(2-methylquinolin-4-ylmethoxy)phenyl group to derive a TACE inhibitor (**3**). Compound **3** was modeled and found to fit into the active site of TACE, with the 4-(2-methylquinolin-4-ylmethoxy)phenyl group occupying the deep S1′ site, while keeping the pyrimidinetrione in a binding mode similar to that observed in the MMP-3 structure. This letter discloses the synthesis and evaluation of **3** and related compounds as TACE inhibitors.

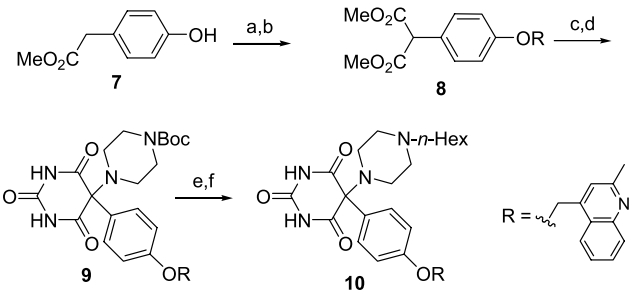
The synthesis of **3** started from phenylacetate **4** (Scheme 1). Carbomethoxylation using NaH and dimethyl carbonate provided the desired diester, which was alkylated with MeI and NaH to give di-substituted malonate **5** after removal of the benzyl group. The phenol moiety in **5** was treated with 4-chloromethyl-2-methylquinoline and Cs_2CO_3 in DMSO to yield **6**. Reaction of **6** with urea and NaOMe in methanol at reflux provided pyrimidine-2,4,6-trione **3**. Several analogues with different R groups were synthesized using a similar sequence.

A series of 5-(piperazin-1-yl)pyrimidine-2,4,6-triones was synthesized using a route highlighted in Scheme 2. Phenylacetate **7** was first converted to the corresponding quinolinylmethyl ether using the Cs_2CO_3 conditions. Carbomethoxylation provided diester **8**, which was converted to pyrimidinetrione with urea and NaOEt. Bromination and displacement of the resulting bromide in situ was effected with Br_2 and mono-Boc-protected piperazine in the presence of K_2CO_3. The Boc protecting group was then removed and an *n*-hexyl group was incorporated by reductive alkylation with hexanal to complete the synthesis of **10**. Pyrimidinetrione **9** was used as a common intermediate to synthesize a number of functionalized piperazine analogues.

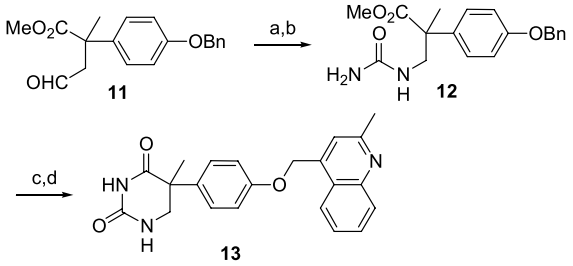
A pyrimidine-2,4-dione analogue (**13**) was also prepared (Scheme 3). Aldehyde **11**, the synthesis of which was



Scheme 1. Reagents and conditions: (a) NaH, Me_2CO_3, THF, at reflux (80%); (b) NaH, MeI, THF, at reflux (81%); (c) H_2, $Pd(OH)_2$/C, MeOH (100%); (d) 4-chloromethyl-2-methylquinoline, Cs_2CO_3, DMSO (95%); (e) NaOMe, urea, MeOH, at reflux (20%).



Scheme 2. Reagents and conditions: (a) 4-chloromethyl-2-methylquinoline, Cs_2CO_3, DMSO (72%); (b) NaH, Me_2CO_3, THF, at reflux (78%); (c) NaOMe, urea, MeOH, at reflux; (d) K_2CO_3, Br_2, 1-Boc-piperazine (45% for two steps); (e) CF_3CO_2H, CH_2Cl_2 (100%); (f) *n*-$C_5H_{11}CHO$, $NaBH(OAc)_3$, *i*-Pr_2NEt, $ClCH_2CH_2Cl$ (63%).

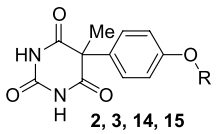


Scheme 3. Reagents and conditions: (a) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, *t*-BuOH, THF, H_2O (91%); (b) DPPA, Et_3N, benzene, NH_3, at reflux (85%); (c) H_2, $Pd(OH)_2$/C, MeOH (100%); (d) 4-chloromethyl-2-methylquinoline, Cs_2CO_3, DMSO (28%).

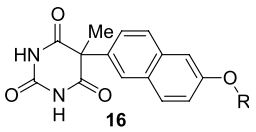
reported previously,[5] was oxidized to a carboxylic acid, and converted to urea **12** after a Curtius rearrangement in the presence of ammonia. The benzyl protecting group was removed via hydrogenolysis, and the resulting phenol reacted with 4-chloromethyl-2-methylquinoline and Cs_2CO_3 in DMSO. Notably, the basic conditions required for the etherification reaction also induced a concomitant intramolecular cyclization to afford the desired pyrimidinedione **13** in one pot in 28% yield (not optimized).

The inhibitory activity was evaluated using porcine TACE (pTACE), as a result of its availability and homology to human TACE.[9] Selectivity was evaluated using MMP-2, -9, and -13 as representatives with deep S1′ pockets and MMP-1 as a representative with shallow S1′.[9] Compounds were also assayed against aggrecanase, a member of ADAMTS family.[10] We were encouraged to find that combination of 4-(2-methylquinolin-4-ylmethoxy)phenyl group with the pyrimidinetrione resulted in a 1 μM inhibitor of pTACE (**3**, Table 1). Compound **3** was inactive at 10 μM in the MMP-1, -2, -9, and -13 assays and at 1 μM in the aggrecanase assay, probably because the 4-(2-methylquinolin-4-ylmethoxy)phenyl group is too wide to fit the S1′ pockets of these enzymes. In contrast, compound **2**, possessing the phenoxyphenyl group (a P1′ well-known to favor MMP binding), was reported to have activity under 100 nM for MMP-2, -9, and -13.[8] Replacing the 2-methylquinolin-4-ylmethoxy group in **3** with 4-hy-

Table 1. In vitro potency of **2**, **3**, and **14–16** in pTACE, MMP-1, -2, -9, -13, and aggrecanase



2, 3, 14, 15



16

	R	pTACE IC_{50}, μM	MMP-1 K_i, μM	MMP-2 K_i, μM	MMP-9 K_i, μM	MMP-13 K_i, μM	Aggrecanase % inh at 1 μM
3	(2-Methylquinolin-4-yl)methyl	1.03	>4.95	>3.33	>2.13	>5.02	0
2	Phenyl	—[a]	—[a]	0.08[b]	0.052[b]	0.065[b]	—[a]
14	H	>100	>4.95	2.53	0.85	>5.02	0
15	Benzyl	>100	>4.95	0.60	>2.13	4.02	0
16	(2-Methylquinolin-4-yl)methyl	>100	—[a]	>3.33	—[a]	—[a]	1

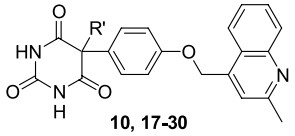
[a] Not tested.
[b] Reported data (Ref. 8a).

droxy and 4-benzyloxy groups resulted in a loss of pTACE activity and restored some MMP activity (**14** and **15**). The sum of these data is consistent with our TACE model. Attempt to replace the central benzene ring in **3** with a 2,6-naphthalene (**16**) resulted in loss of TACE activity.

Based on the SAR of pyrimidinetrione-derived MMP inhibitors[8] and our computer model of TACE, we sought to improve TACE potency of **3** by replacing the methyl group at the 5-position of the pyrimidinetrione with a variety of substituents (Table 2). Piperidine (**17**), morpholine (**18**), and piperazine (**19**) analogues gave essentially no improvement in pTACE potency. Methylation on the second nitrogen of the piperazine (**20**) resulted in a 91 nM inhibitor of pTACE, a 12-fold increase compared to the des-Me analogue **19**. Compound **20** still maintained selectivity over the 4 MMPs and aggrecanase. Many other alkyl groups, including isopropyl, *n*-hexyl, neopentyl, benzyl, 2-phenylethyl, and 3-phenylpropyl groups (**10**, **21–25**), gave TACE inhibitors with similar potency, whereas a more rigid 4-nitrophenyl analogue **26** is less active (2.8 μM). Amide (**27** and **28**), sulfonamide (**29**), and carbamate (**30**) analogues were found to offer no advantage. Unlike the selectivity observed with the majority of analogues, **24** and **26** displayed sub-micromolar K_i's for MMP-2, -9, and -13. A plausible explanation is that the symmetrical pyrimidinetrione core can rotate 180° around the urea carbonyl to project the phenethyl and 4-nitrophenyl groups into the deep S1′ pockets of the three MMPs.[11] The other R′ groups in Table 2 are probably not as complementary to the MMP S1′ sites.

We also briefly investigated the effects of other heterocycles in place of the pyrimidinetrione. Compared to pyrimidinetrione **3**, hydantoin analogue **31**[12] was about 3-fold less active for pTACE (Table 3). Cyanuric acid **32**[13] was even less effective, resulting in a 7-fold loss of activity compared to **3**. Pyrimidinedione **13** is approximately 4-fold less active than **3**. The reduced activity of the pyrimidinedione could be due to the higher

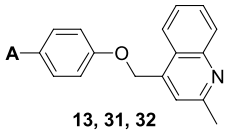
Table 2. In vitro potency of **10** and **17–30** in pTACE, MMP-1, -2, -9, -13, and aggrecanase



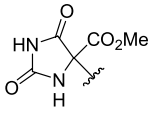
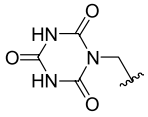
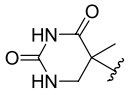
10, 17-30

	R′	pTACE IC_{50}, μM	MMP-1 K_i, μM	MMP-2 K_i, μM	MMP-9 K_i, μM	MMP-13 K_i, μM	Aggrecanase % inh at 1 μM
17	Piperidin-1-yl	0.855	>4.95	>3.33	>2.13	>5.02	0
18	Morpholin-4-yl	0.535	>4.95	>3.33	>2.13	>5.02	1
19	Piperazin-1-yl	1.10	>4.95	>3.33	>2.13	>5.02	0
20	4-Me-piperazin-1-yl	0.091	>4.95	>3.33	>2.13	>5.02	37
21	4-*i*-Pr-piperazin-1-yl	0.096	>4.95	>3.33	>2.13	>5.02	0
10	4-*n*-Hex-piperazin-1-yl	0.081	>4.95	>3.33	>2.13	>5.02	12
22	4-*neo*-Pent-piperazin-1-yl	0.160	>4.95	>3.33	>2.13	>5.02	10
23	4-Bn-piperazin-1-yl	0.195	>4.95	>3.33	>2.13	>5.02	5
24	4-[$Ph(CH_2)_2$]-piperazin-1-yl	0.084	>4.95	0.083	0.085	0.095	38
25	4-[$Ph(CH_2)_3$]-piperazin-1-yl	0.110	>4.95	3.10	2.04	>5.02	12
26	4-(4-NO_2-Ph)-piperazin-1-yl	2.80	4.35	0.116	0.138	0.356	0
27	4-Ac-piperazin-1-yl	0.620	>4.95	>3.33	>2.13	>5.02	8
28	4-Piv-piperazin-1-yl	0.540	>4.95	>3.33	>2.13	>5.02	0
29	4-Ms-piperazin-1-yl	0.275	>4.95	>3.33	>2.13	>5.02	5
30	4-Boc-piperazin-1-yl	0.160	>4.95	>3.33	>2.13	>5.02	10

Table 3. In vitro potency of **13**, **31**, and **32** in pTACE



13, 31, 32

	A	pTACE IC_{50}, μM
31[a]		4.70
32		7.30
13[a]		3.70

[a] Tested as a racemic mixture.

enthalpic cost to enolize, as the pyrimidinetrione was shown to exist as an enol tautomer when binding to MMPs.[8]

In summary, using a quinoline P1′ group that was previously optimized in our laboratories for TACE, we successfully converted pyrimidine-2,4,6-trione MMP inhibitors to TACE inhibitors. Modification of the 5-phenylpyrimidine-2,4,6-trione template led to identification of several inhibitors with IC_{50}s of 100 nM for porcine TACE and selectivity against MMP-1, -2, -9, -13, and aggrecanase.

Acknowledgments

The authors thank Bin Jiang for assistance in synthesis of compound **16**, John Giannaras and Paul Strzemienski for assistance in enzymatic assays, and Dr. James E. Sheppeck II for a critical review of the manuscript.

References and notes

1. Bemelmans, M. H. A.; van Tits, L. J. H.; Buurman, W. A. *Crit. Rev. Immunol.* **1996**, *16*, 1.
2. Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 2295.
3. (a) Moss, M. L.; White, J. M.; Lambert, M. H.; Andrews, R. C. *Drug Discovery Today* **2001**, *6*, 417; (b) Black, R. A. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 1.
4. For a recent review of TACE inhibitors, see: Skotnicki, J. S.; Levin, J. I. *Annu. Rep. Med. Chem.* **2003**, *38*, 153.
5. Duan, J. J.-W.; Chen, L.; Wasserman, Z. R.; Lu, Z.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Hardman, K. D.; Magolda, R. L.; Newton, R. C.; Christ, D. D.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem.* **2002**, *45*, 4954.
6. (a) Wasserman, Z. R.; Duan, J. J.-W.; Voss, M. E.; Xue, C.-B.; Cherney, R. J.; Nelson, D. J.; Hardman, K. D.; Decicco, C. P. *Chem. Biol.* **2003**, *10*, 215; (b) Cherney, R. J.; Duan, J. J.-W.; Voss, M. E.; Chen, L.; Wang, L.; Meyer, D. T.; Wasserman, Z. R.; Hardman, K. D.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Mandlekar, S.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem.* **2003**, *46*, 1811; (c) Duan, J. J.-W.; Lu, Z.; Xue, C.-B.; He, X.; Seng, J. L.; Roderick, J. J.; Wasserman, Z. R.; Liu, R.-Q.; Covington, M. B.; Magolda, R. L.; Newton, R. C.; Trzaskos, J. M.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2035.
7. For a review of MMP inhibitors, see: Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2001**, *8*, 425.
8. (a) Foley, L. H.; Palermo, R.; Dunten, O.; Wang, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 969; (b) Grams, F.; Brandstetter, H.; D'Alo, S.; Geppert, D.; Krell, H.-W.; Leinery, H.; Livi, V.; Menta, E.; Oliva, A.; Zimmermann, G. *Biol. Chem.* **2001**, *382*, 1277; (c) Dunten, P.; Kammlott, U.; Crowther, R.; Levin, W.; Foley, L. H.; Wang, P.; Palermo, R. *Protein Sci.* **2001**, *10*, 923; (d) Brandstetter, H.; Grams, F.; Glitz, D.; Lang, A.; Huber, R.; Bode, W.; Krell, H.-W.; Engh, R. A. *J. Biol. Chem.* **2001**, *276*, 17405; (e) Grams, F.; Brandstetter, H.; Engh, R. A.; Glitz, D.; Krell, H.-W.; Livi, V.; Menta, E.; Moroder, L.; Muller, J. C. D.; Roedern, E. G.; Zimmermann, G. In *Matrix Metalloproteinase Inhibitors in Cancer Therapy*; Clendeninn, N. J., Appelt, K., Eds.; Humana: New Jersey, 2001, pp 223–243.
9. For description of assay protocols, see: Xue, C.-B.; Voss, M. E.; Nelson, D. J.; Duan, J. J.-W.; Cherney, R. J.; Jacobson, I. C.; He, X.; Roderick, J.; Chen, L.; Corbett, R. L.; Wang, L.; Meyer, D. T.; Kennedy, K.; DeGrado, W. F.; Hardman, K. D.; Teleha, C. A.; Jaffee, B. D.; Liu, R.-Q.; Copeland, R. A.; Covington, M. B.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *J. Med. Chem.* **2001**, *44*, 2636.
10. Miller, J. A.; Liu, R.-Q.; Davis, G. L.; Pratta, M. A.; Trzaskos, J. M.; Copeland, R. A. *Anal. Biochem.* **2003**, *314*, 260.
11. Sawa, M.; Kondo, H.; Nishimura, S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 581.
12. Compound **31** was isolated as a byproduct during conversion of **8** to **9**, presumably because only one of the ester groups reacted with urea in the urea/NaOEt step and subsequently cyclized under K_2CO_3/Br_2 conditions.
13. Synthesis of **32**: (a) 4-(hydroxymethyl)phenol, 4-chloromethyl-2-methylquinoline, Cs_2CO_3, DMSO (81%); (b) cyanuric acid, DEAD, PPh_3, DMF (14%).