Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1577–1582
Bioorganic & Medicinal Chemistry Letters

Potent, exceptionally selective, orally bioavailable inhibitors of TNF-α Converting Enzyme (TACE): Novel 2-substituted-1*H*-benzo[*d*]imidazol-1-yl)methyl)benzamide P1′ substituents

Gregory R. Ott,* Naoyuki Asakawa, Zhonghui Lu, Rajan Anand, Rui-Qin Liu, Maryanne B. Covington, Krishna Vaddi, Mingxin Qian, Robert C. Newton, David D. Christ, James M. Trzaskos and James J.-W. Duan

Departments of Discovery Chemistry and Discovery Biology, Bristol-Myers Squibb Research and Development, Rte 206 and Province Line Road, Princeton, NJ 08543, USA

Received 17 December 2007; revised 17 January 2008; accepted 18 January 2008
Available online 26 January 2008

Abstract—Novel ((2-substituted-1*H*-benzo[*d*]imidazol-1-yl)methyl)benzamides were found to be excellent P1′ substituents in conjunction with unique constrained β-amino hydroxamic acid scaffolds for the discovery of potent selective inhibitors of TNF-α Converting Enzyme (TACE). Optimized examples proved potent for TACE, exceptionally selective over a wide panel of MMP and ADAM proteases, potent in the suppression of LPS-induced TNF-α in human whole blood and orally bioavailable.

The overexpression of the pro-inflammatory cytokine Tumor Necrosis Factor-α (TNF-α) has been implicated in numerous pathological conditions.[1] The anti-TNF-α biological therapeutics entaneracept, infliximab, and adalimumab have demonstrated clinical success in inflammatory and autoimmune diseases and as such have validated the modulation of TNF-α as a drug discovery paradigm.[2] TNF-α Converting Enzyme (TACE or ADAM-17) is the principle sheddase that governs the cleavage of membrane bound pro-TNF-α to the soluble form.[3] TACE is a member of the ADAM (A Disintegrin And Metalloprotease) family in the metzincin superfamily of metalloproteases. We and others have targeted TACE for the development of orally administered, small molecule modulators of TNF-α.[4] In particular, structural motifs including γ-lactam[5] (**1**, Fig. 1), cyclic succinate[6] (**2**), β,β-disubstituted-β-amino[7] (**3**), α,β-cyclized-β-amino[8] (**4**, **5**), and β-sulfone[9] (**6**) hydroxamic acids have been shown to inhibit TACE as well as modulate TNF-α production in both cell-based assays and animal models. Common binding elements among these inhibitors include the classical bidentate coordination to the active site zinc by the hydroxamic acid, hydrogen bonds from the hydroxamic acid NH and OH to the protein backbone as well as a key hydrogen bond acceptor from the amide carbonyl or sulfone. Common among this group is the 4-(2-quinolinylmethoxy)phenyl P1′ substituent.

This unique 4-(2-quinolinylmethoxy)phenyl P1′ element provided selectivity against the structurally related matrix metalloproteases (MMPs); selectivity against the MMPs was desired since broad based MMP inhibitors were shown to have musculoskeletal side effects in clinical trials.[10] The P1′ component was integral to achieving potency in a cellular assay which measures the suppression of LPS-induced TNF-α in human whole blood (WBA).[11] Acceptable oral pharmacokinetic profiles were also achieved on examples containing this moiety. Importantly, this group translated across scaffolds with a high degree of success imparting similar properties to each. Though important for achieving selectivity against related MMP and ADAM proteases, there was still room for improvement in this area especially against MMPs-3,-7,-8, and -12. Thus, the goals for the next generation of TACE inhibitors required increased selectivity against MMPs and further structural diversification

Keywords: TACE; TNF-α Converting Enzyme; MMP; Matrix metalloprotease; TNF modulator.
* Corresponding author at present address: Cephalon, Inc. 145 Brandywine Parkway, West Chester, PA 19380, USA. Tel.: +1 610 738 6861; fax: +1 610 738 6558; e-mail: gott@cephalon.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.075

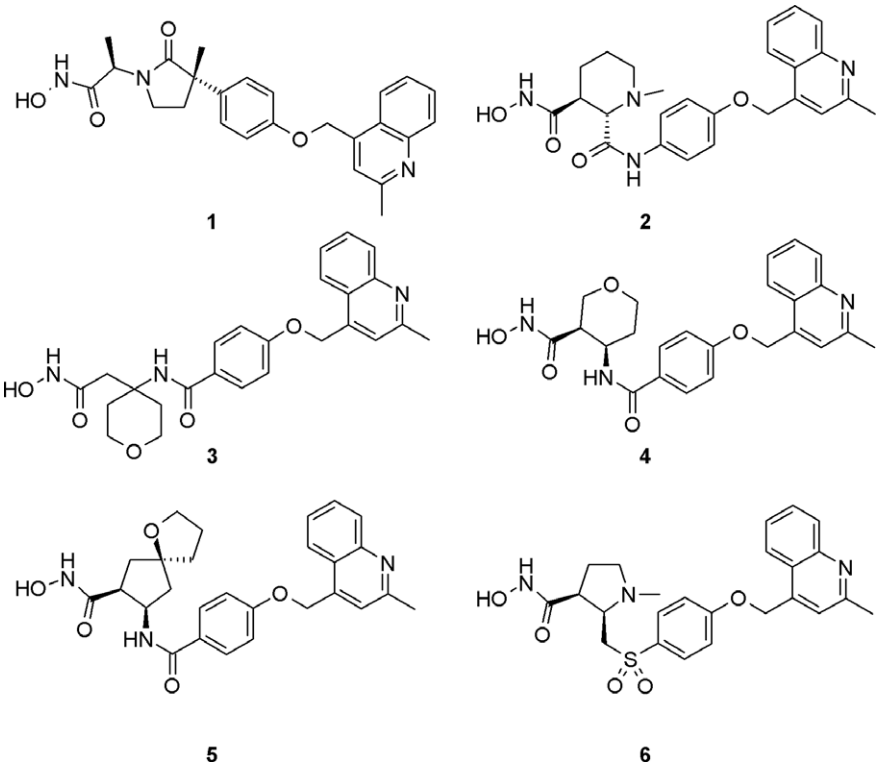


Figure 1. Scaffold translation of 4-(2-methyl-quinolinylmethoxy)phenyl P1′ group.

that included replacement of the 4-(2-methyl-quinolinyl)methoxy substituent while retaining cell potency and acceptable oral pharmacokinetics.

Toward this end, reexamination of the P1′ moieties from the γ-lactam scaffold studies revealed examples that demonstrated suitable attributes such as cell permeability, selectivity, and potential for increased structural diversity. Of note, γ-lactam **7** (Fig. 2), containing the 2-(methylthio)benzimidazolemethyl phenyl moiety, stood out as exhibiting cell potency (WBA IC_{50} = 316 nM) and modest selectivity against MMPs-1,-2, and -9. The benzimidazole group was especially attractive for drug-like properties, ease of incorporation, and numerous positions to integrate structural changes.

The recently discovered *cis*-α,β-substituted-β-benzamido hydroxamic acid scaffolds were characterized by good TACE potency/selectivity profiles and optimized examples proved to have excellent oral pharmacokinetic properties.[8] We envisioned the 2-substituted-1*H*-benzo[*d*]imidazol-1-yl)methyl)benzamide P1′ substituents would provide a promising commencement to further optimize the factors listed above.

Thus, alkylation of 2-methylthio[1*H*]benzimidazole with methyl 4-(bromomethyl)benzoate provided ester **10** (Scheme 1). Saponification and coupling to the BOC-protected pyrrolidine **12**[8] provided the amide **13**. Deprotection and derivatization at this stage were followed by conversion to the hydroxamic acid. This chemistry was applicable to all analogues synthesized throughout this study.[12]

The initial examples were tested in vitro using semi-purified porcine TACE (pTACE) to evaluate enzyme

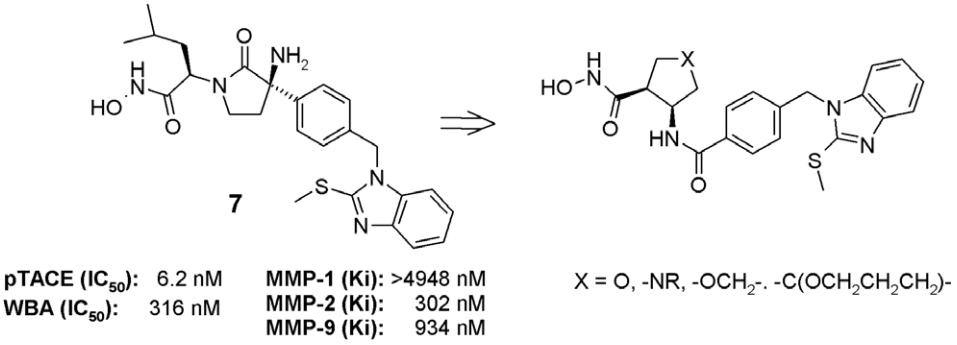
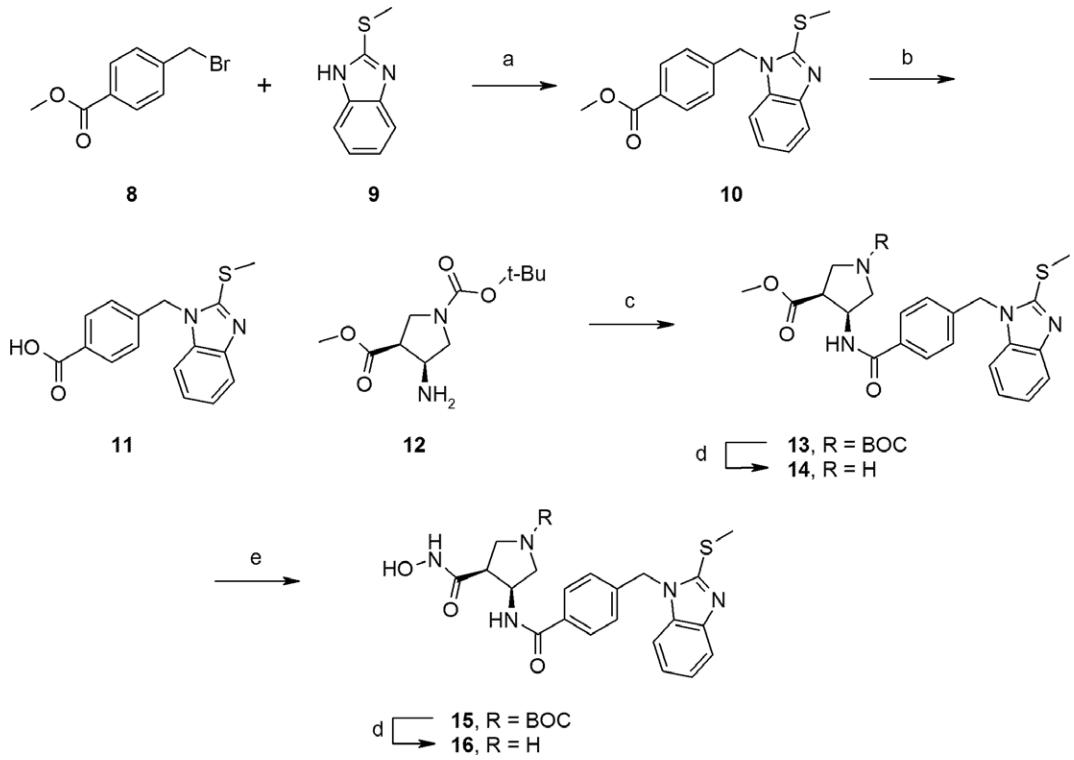


Figure 2. ((2-Substituted-1*H*-benzo[*d*]imidazol-1-yl)methyl)phenyl P1′ moieties.



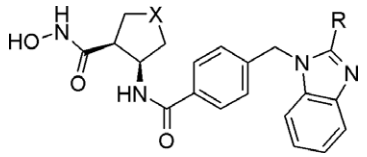
Scheme 1. Reagents: (a) Cs_2CO_3, DMSO (99%); (b) LiOH, MeOH, H_2O (72%); (c) BOP Reagent, DIPEA, DMF (99%); (d) TFA, CH_2Cl_2 (99%); (e) NH_2OH, NaOMe, MeOH.

potency.[11] The primary selectivity profile was evaluated against MMPs-1,-2, and -9. Cellular suppression of LPS-induced TNF-α was measured using human whole blood (WBA). Membrane permeability [P_{app} ($A \rightarrow B$)], was measured using Caco-2 cells.[13] Pharmacokinetic studies were done in a discrete or cassette-dose fashion (*n*-in-1) administered intravenously and orally to Sprague–Dawley rats and beagle dogs; the plasma samples were analyzed by LC/MS/MS.[14]

We were gratified to find that the 2-substituted benzimidazolyl P1′ substituents translated well to the β-amino acid scaffolds (Table 1); analogues **15**–**20** displayed potent activity against pTACE with IC_{50}s ranging between <1 and 2.6 nM. Interestingly, pTACE potency was unaffected by increasing steric bulk at the 2-position of the benzimidazole. Improved selectivity was observed against MMP-2 and -9, as compared to the γ-lactam **7**. Importantly, potent activity in the cellular assay was realized (WBA IC_{50}s <100 nM). Caco-2 permeability values for these inhibitors suggested low potential for oral absorption with $P_{app} \leqslant 0.2 \times 10^{-6}$ cm/s. Indeed, pharmacokinetic studies in rats revealed less than 1% oral bioavailability for **16** and **20**.

With the realization of excellent enzyme and cell potency for these analogues, further profiling against a wider panel of MMPs was undertaken (Table 2).

Table 1. In vitro properties of inhibitors **15**–**20**



Compound	X	R	pTACE[a] (IC_{50} nM)	WBA[b] (IC_{50} nM)	MMP-1,-2,-9 K_i (nM)	Caco-2[c]
15	NBOC	SCH_3	<1.0	62	>2128	0.2
16	NH	SCH_3	1.6	34	>2128	0.2
17	NBOC	CH_3	1.0	92	>2128	0.1
18	NH	CH_3	2.6	56	>2128	0.1
19	NBOC	*i*-Pr	1.0	70	>2128	0.2
20	NH	*i*-Pr	1.6	64	>2128	0.1

[a] pTACE IC_{50} and MMP K_i values are from single determination.
[b] Inhibition of TNF-α release in WBA was determined with three donors.
[c] P_{app} ($A \rightarrow B$) $\times 10^{-6}$ cm/s.

Table 2. MMP profile of inhibitors **15**, **17**, and **19**

Enzyme[a]	**15**	**17**	**19**
TACE[b]	<1	1.0	1.0
MMP-1	>4949	>4946	>4946
MMP-2	>3333	>3333	>3333
MMP-3	>4501	2609	3915
MMP-7	4075	5858	3502
MMP-8	1957	1865	>3058
MMP-9	>2128	>2128	>2128
MMP-10[b]	>10,000	1600	4600
MMP-12[b]	540	250	390
MMP-13	>5025	>5025	>5025
MMP-14	>5290	>5290	>5290
MMP-15	>7088	>7088	>7088
MMP-16	>5454	>5554	>5554

[a] K_i (nM).
[b] IC_{50} (nM).

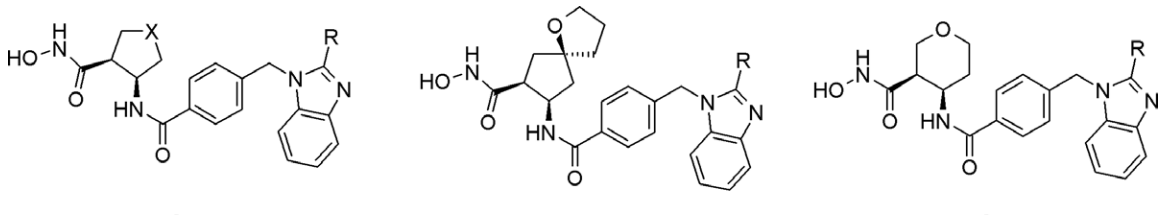
Representative of this class, inhibitors **15**, **17**, and **19** displayed exceptional selectivity against the panel of MMPs. Notably, superb selectivity over MMP-3,-7,-8, and -12 was achieved; selectivity over this subset was, in general, modest with the 4-(2-methylquinolinyl)methoxyphenyl P1′.

To further evaluate in vitro and in vivo properties with the new benzimidazole P1′ motif, a more thorough exploration of β-benzamido hydroxamic acid scaffolds was initiated. These scaffolds were effective for constructing cell potent, orally bioavailable TACE inhibitors. The β-benzamido hydroxamic acid scaffolds of particular interest included the cyclopentane[8a] (Table 3, **A**, X=CH_2), pyrollidine[8b] (**A**, X=*N*-alkyl), tetrahydrofuran[8b] (**A**, X=O), oxaspiro[4.4]nonane[15] (**B**), and tetrahydopyran[8a] (**C**).

In conjunction with the cyclopentane-β-benzamido hydroxamic acid, we were pleased to find that both the isopropyl and cyclopropyl derivatives **21** and **22** retained potency in both the enzymatic and cellular assays. Consistent with the improvement in permeability in the Caco-2 assay as well as the more modest clearance, the cyclopropyl derivative **22** displayed better oral bioavailability (22%) as compared to isopropyl derivative **21** (8%). The *N*-(2-propynyl)-pyrrolidine analogues **23** and **24** gave favorable potency in the enzyme assay, though we observed a drop in cell potency that appeared to coincide with the bulkier *tert*-butylbenzimidazole derivative **24**. Minimal oral bioavailability was observed for **23** and **24** (3% and 8%, respectively) with higher intrinsic clearance and low permeability contributing to these results.

Turning to the oxaspiro[4.4]nonane scaffold, the isopropyl derivative **25** maintained cell potency (WBA IC_{50} = 69 nM) and provided acceptable oral bioavailability (F = 22%), though this was in contrast to both the Caco-2 value and the higher clearance. Unlike the corresponding analogue on the cyclopentyl scaffold, the cyclopropylbenzimidazole analogue **26** did not offer any advantage over the isopropyl derivative. Turning toward larger and more lipophilic benzimidazole substituents, the *tert*-butyl derivative **27** gave better oral bioavailability (F = 29%), which, in this instance, was consistent with the enhanced Caco-2 value; again, a slight erosion in cell potency was observed (WBA

Table 3. In vitro and in vivo results for **21**–**33**



A **B** **C**

Compound	Scaffold A,B,C	X	R	pTACE[a] (IC_{50} nM)	WBA[b] (IC_{50} nM)	MMP-1,-2,-9 K_i (nM)	Caco-2[c]	Cl[d](L/h/kg)	F[d] (%) rat
21	A	CH_2	*i*-Pr	1.0	65	>4948, >3333, >2128	0.1	3.0	8
22	A	CH_2	*c*-Pr	2.7	54	>4948, >3333, >2128	2.0	1.5	22
23	A	N(2-propynyl)	*i*-Pr	1.8	137	>4948, >3333, >2128	0.1	9.3	3
24	A	N(2-propynyl)	*t*-Bu	2.3	317	>4948, >3333, >2128	0.4	6.8	8
25	B	—	*i*-Pr	1.1	69	>4948, >3333, >2128	0.1	6.9	22
26	B	—	*c*-Pr	1.3	42	>4948, >3333, >2128	0.1	1.4	14
27	B	—	*t*-Bu	2.0	252	>4948, >3333, >2128	1.4	2.9	29
28	B	—	$CF(Me)_2$	<1.0	140	>4948, >3333, >2128	2.4	2.4	33
29	C	—	*i*-Pr	1.6	105	>4948, >3333, >2128	0.5	3.8	8
30	C	—	$CF(Me)_2$	2.2	153	>4948, >3333, >2128	0.3	1.9	36
31	C	—	CF_2CH_3	2.6	188	>4948, >3333, >2128	0.7	1.2	55
32	C	—	CF_3	1.4	232	>4948, >3333, >2128	0.7	1.6	53
33	A	O	CF_3	1.0	102	>4948, >3333, >2128	0.1	3.2	20

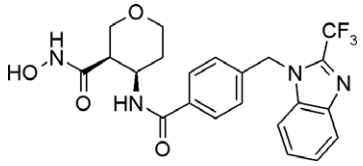
[a] pTACE IC_{50} and MMP K_i values are from single determination.
[b] Inhibition of TNF-α release in WBA was determined with three donors.
[c] P_{app} $(A \rightarrow B) \times 10^{-6}$ cm/s.
[d] Determination of 3 for each dosing group, avg. value.

IC_{50} = 252 nM). In an effort to maintain cell potency and provide enough lipophilicity to boost oral absorption, addition of a fluorine atom in place of the methine hydrogen of the isopropyl provided **28**. Importantly, a boost to the Caco-2 permeability was observed and indicative of that oral bioavailability improved to 33%.

The previously described tetrahydropyranyl and tetrahydrofuranyl scaffolds were shown to possess favorable potency/pharmacokinetic properties;[8] this trend continued with the benzimidazole P1′ substituents. Relative to the prior analogues, improved clearance values, though still in the moderate range, were observed for **30**–**32** (1.2–1.9 L/h/kg). The fluoroisopropyl analogue **30** displayed favorable cell potency (WBA IC_{50} = 153 nM) with good oral bioavailability in rat (F = 36%). It appeared that substituting fluorine for hydrogen in the 2-alkyl position of the benzimidazole led to favorable pharmacokinetics without sacrificing potency or selectivity. Indeed, retention of cell potency and a boost in oral bioavailability were observed for both the difluoroethylbenzimidazole derivative **31** (WBA IC_{50} = 188 nM, F = 55%) and the trifluoromethyl derivative **32** (WBA IC_{50} = 232 nM, F = 53%). The tetrahydrofuranyl derivative **33** proved to be more potent in the WBA (IC_{50} = 102 nM) as compared to **32** but this resulted in a less favorable pharmacokinetic profile (F = 20%). Interestingly, for the tetrahydropyranyl analogues, increasing oral bioavailability paralleled increasing Caco-2 values, though the P_{app} values were modest in magnitude.

Inhibitor **32** was profiled against a wider panel of MMP and ADAM proteases (Table 4). Importantly, **32** displayed outstanding selectivity, especially against MMP-3,-7,-12, and ADAMTS-4 and -5.

Table 4. MMP and ADAM profile of inhibitor **32**



Enzyme[a]	K_i (nM)
pTACE (IC_{50})	1.4
MMP-1	>10,000
MMP-2	>10,000
MMP-3	>10,000
MMP-7	>10,000
MMP-8	>10,000
MMP-9	>10,000
MMP-10 (IC_{50})	>10,000
MMP-12 (IC_{50})	8000
MMP-13	>10,000
MMP-14	>10,000
MMP-15	>10,000
MMP-16	>10,000
ADAMTS-1	>1000
ADAMTS-4	6700
ADAMTS-5	1000
ADAM-10	>10,000

[a] pTACE IC_{50} and MMP K_i values are from single determination.

Table 5. Pharmacokinetic parameters of **32**[a]

	PK parameters	Rat	Dog
IV	Dose (mg/kg)	5.0	2.0
	$t_{1/2}$ (h)	3.5	4.2
	Cl (L/h/kg)	1.6	0.6
	V_{ss} (L/kg)	1.5	1.0
	AUC (nM h)	6849	9967
PO	Dose (mg/kg)	8.0	8.0
	t_{max} (h)	0.3	0.5
	$t_{1/2}$ (h)	2.4	4.8
	AUC (nM h)	5840	39,466
	F (%)	53	99

[a] Determination of 3 for each dosing group, avg. value.

Further in vivo profiling for **32** in a second species (dog) was performed and the full pharmacokinetic profile in both rat and dog is shown in Table 5. Following iv and oral dosing in rats, **32** is exemplified by low to moderate clearance (1.6 L/h/kg), rapid absorption (t_{max} = 0.3 h), and high oral exposure (AUC = 5840 nMh). The oral bioavailability is 53%. Pharmacokinetic analysis in beagle dogs showed lower clearance (0.6 L/h/kg) as compared to rats and longer oral half-life ($t_{1/2}$ = 4.8 h). Near complete oral bioavailability (F = 99%) was observed in dogs.

Compound **32**, when analyzed for serum protein binding, displayed acceptable free fraction in rodents (rat, 20% unbound) as well as higher order species, (dog, 20% unbound, human, 21% unbound).[16]

Oral efficacy of **32** was determined using the LPS-TNF model of endotoxemia in mouse.[11] Lipopolysaccharide was administered along with different doses of **32** in vehicle, by oral gavage administration at t = 0 h. One hour post dose, the mice were euthanized, whole blood collected by cardiac puncture, and the plasma used for the measurement of TNF-α concentration. **32** displayed a dose dependent suppression of TNF-α with a calculated ED_{50} 1.9 mg/kg.

In summary, novel ((2-substituted-1*H*-benzo[*d*]imidazol-1-yl)methyl)benzamides were found to be excellent P1′ substituents in conjunction with unique β-amino hydroxamic scaffolds for the discovery of potent, selective inhibitors of TACE. Optimized examples demonstrated oral bioavailability. In particular, **32** proved potent for pTACE, selective over a wide panel of MMPs and ADAM proteases as well as potent in the suppression of LPS-induced TNF-α in human whole blood. Importantly, following oral dosing, **32** displayed an ED_{50} of 1.9 mg/kg in an acute model of inflammation.

Acknowledgments

We thank John Giannaras, Sherrill Nurnberg and Paul Strzemienski for running in vitro assays, Robert J. Collins for LPS study in animals, Bernice Brogdon, Bing Lu, Hang Zheng and Jingtao Wu for assistance in pharmacokinetic studies and Maria Ribadeneira for Caco-2 assay. We thank George Emmett and Joerg Deerberg for providing synthetic intermediates.

References and notes

1. Newton, R. C.; Decicco, C. P. *J. Med. Chem.* **1999**, *42*, 2295.
2. (a) Moreland, L. W.; Baumgartner, S. W.; Schiff, M. H.; Tindall, E. A.; Fleischmann, R. M.; Weaver, A. L.; Ettlinger, R. E.; Cohen, S.; Koopman, W. J.; Mohler, K.; Widmer, M. B.; Blosch, C. M. *N. Engl. J. Med.* **1997**, *337*, 141; (b) Lipsky, P. E.; van der Heijde, D. M. F. M. E.; St. Clair, W.; Furst, D. E.; Breedveld, F. C.; Kalden, J. R.; Smolen, J. S.; Weisman, M.; Emery, P.; Feldmann, M.; Harriman, G. R.; Maini, R. N. *N. Engl. J. Med.* **2000**, *343*, 1594; (c) Machold, K. P.; Smolen, J. S. *Exp. Opin. Bio. Ther.* **2003**, *3*, 351.
3. Black, R. A. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 1.
4. For a recent review see Skotnicki, J. S.; Levin, J. I. *Ann. 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.; Wexlre, R. R.; Decicco, C. P. *J. Med. Chem.* **2002**, *45*, 4954.
6. (a) Xue, C.-B.; He, X.; Roderick, J.; Corbett, R. L.; Duan, J. J.-W.; Liu, R.-Q.; Covington, M. B.; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4293; (b) Xue, C.-B.; He, X.; Roderick, J.; Corbett, R. L.; Duan, J. J.-W.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Ribadeneira, M. D.; Vaddi, K.; Christ, D. D.; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Wexler, R. R.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4299.
7. Gilmore, J. L.; King, B. W.; Harris, C.; Maduskuie, T.; Mercer, S. E.; Liu, R.; Covington, M. B.; Qian, M.; Ribadeneria, M. D.; Vaddi, K.; Trzaskos, J. M.; Newton, R. C.; Duan, J. J.-W.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2699.
8. (a) Duan, J. J.-W.; Chen, L.; Lu, Z.; Xue, C.-B.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Wasserman, Z. R.; Vaddi, K.; Christ, D. D.; Trzaskos, J. M.; Newton, R. C.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 241; (b) Ott, G. R.; Asakawa, N.; Lu, Z.; Liu, R.-Q.; Covington, M. B.; Vaddi, K.; Qian, M.; Newton, R. C.; Christ, D. D.; Trzaskos, J. M.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 694.
9. Xue, C.-B.; Chen, X. T.; He, X.; Roderick, J.; Corbett, R. L.; Ghavimi, B.; Liu, R.-Q.; Covington, M. B.; Qian, M.; Ribadeneira, M. D.; Vaddi, K.; Trzaskos, J.; Newton, R. C.; Duan, J. J.-W.; Decicco, C. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4453.
10. Levitt, N. C.; Eskens, F. A. L. M.; O'Byrne, K. J.; Propper, D. J.; Denis, L. J.; Owen, S. J.; Choi, L.; Foekens, J. A.; Wilner, S.; Wood, J. M.; Nakajima, M.; Talbot, D. C.; Steward, W. P.; Harris, A. L.; Verweij, J. *Clin. Cancer Res.* **2001**, *7*, 1912.
11. Details of the pTACE (porcine TACE), MMPs, human whole blood (WBA) assays as well as LPS-mouse efficacy model are reported in previous publication, 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.
12. Unless otherwise noted, all starting materials are commercially available and all synthesized compounds are in accordance with their ^{1}H NMR and mass spectral data. 2-(1,1-difluoro-ethyl)-1*H*-benzo[*d*]imidazole and 2-(1-fluoro-1-methyl-ethyl)-1*H*-benzo[*d*]imidazole used to prepare compounds **28**, **30**, and **31** were synthesized from *o*-phenylenediamine and the corresponding carboxylic acids, 2,2-difluoropropanoic acid, and 2-fluoroisobutyric acid, via two-step procedure involving BOP reagent coupling to form the mono-amide and cyclization with AcOH. For a similar benzimidazole cyclization procedure see Fonseca, T.; Gigante, B.; Gilchrist, T. L. *Tetrahedron* **2001**, *57*, 1793.
13. Artursson, P.; Palm, K.; Luthman, K. *Adv. Drug Delivery Rev.* **1996**, *22*, 67.
14. Wu, J.-T.; Zeng, H.; Qian, M.; Brogdon, B. L.; Unger, S. E. *Anal. Chem.* **2000**, *72*, 61.
15. Ott, G. R.; Asakawa, N.; Liu, R.; Covington, M. B.; Qian, M.; Vaddi, K.; Newton, R. C.; Christ, D. D.; Trzaskos, J. M.; Gayla, L.; Scholz, T.; Marshall, M.; Duan, J. J.-W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1288.
16. For details of serum protein binding measurement, see 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.