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Potent, selective, orally bioavailable inhibitors of tumor necrosis factor-α converting enzyme (TACE): Discovery of indole, benzofuran, imidazopyridine and pyrazolopyridine P1′ substituents

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Abstract—Potent and selective inhibitors of tumor necrosis factor-α converting enzyme (TACE) were discovered with several new heterocyclic P1′ groups in conjunction with cyclic β-amino hydroxamic acid scaffolds. Among them, the pyrazolopyridine provided the best overall profile when combined with tetrahydropyran β-amino hydroxamic acid scaffold. Specifically, inhibitor **49** showed IC_{50} value of 1 nM against porcine TACE and 170 nM in the suppression of LPS-induced TNF-α of human whole blood. Compound **49** also displayed excellent selectivity over a wide panel of MMPs as well as excellent oral bioavailability ($F\% > 90\%$) in rat n-in-1 PK studies.

Tumor necrosis factor-α (TNF-α), a cytokine produced by activated macrophages and monocytes, is a principal mediator of inflammatory response. In particular, TNF-α has been shown to be overexpressed in the joints of rheumatoid arthritis patients.[1] The clinical success of anti-TNF-α biologics, such as Enbrel, Remicade and Humira, has validated TNF-α as a drug discovery target.[2] As a result, many approaches to intersect the TNF-α signaling pathway with orally active small molecules are being pursued.[3] One such approach is through the inhibition of TNF-α converting enzyme (TACE), which is responsible for the release TNF-α from cells.[4]

TACE (ADAM-17) is a Zn-dependent metalloproteinase belonging to a subclass of the metzincin superfamily. Due to structure similarities between the active sites of TACE and related matrix metalloproteinases (MMPs), early TACE inhibitors were derived from MMP inhibitors and consequently suffered from broad spectrum activity against the MMP family. Broad-based MMP inhibitors were shown to have musculoskeletal side effects in clinical trials.[5] Recently, inhibitors with selectivity for TACE against MMPs have been reported.[6] Figure 1 shows three representative compounds from our laboratory, γ-lactamhydroxamic acid **1**,[7] tetrahydropyran-β-aminohydroxamic acid **2**,[8] and pyrrolidine-β-aminohydroxamic acid **3**.[9] The commonly shared 4-(2-methylquinolin-4-ylmethoxy)phenyl group was designed and optimized to bind to the S1′ pocket of TACE, which was demonstrated to be the critical

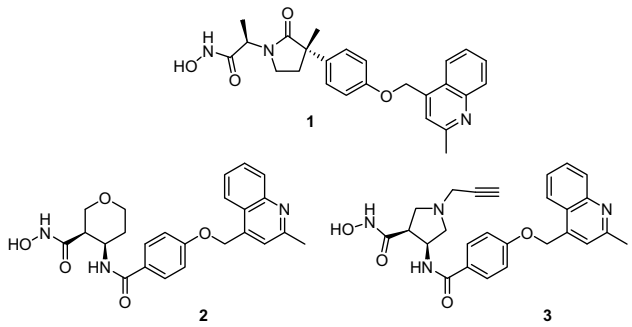

Figure 1. Representative TACE inhibitors from BMS.

Keywords: TACE inhibitors; MMP inhibitors.
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determinant for TACE selectivity.[7] In addition, this group was also found to improve cellular activity and pharmacokinetic profile.

One of the concerns was the potential O-dealkylation in vivo, which would generate phenols that have broad MMP inhibitory activity. Second, although compounds **1**–**3** had excellent selectivity (>500-fold) against most of the MMPs, they all showed some level of activity for MMP-3, -7, -8, -12.[8,9] In our efforts to address these issues, we designed several [5,6] bicyclic hetereocycles (Fig. 2) and evaluated them using tetrahydropyran- and pyrrolidine-derived β-aminohydroxamic acid scaffolds. Because of the critical role of 2-methylquinoline on selectivity, most of this work was focused on 2-substituted [5,6] bicyclic hetereocycles attached to the benzamide at 1 or 3 position. The results from 2-substituted-1*H*-benzo[*d*]imidazole P1′series (**4**) were reported in a separate paper.[10] This paper discloses the preparation and activity of P1′ analogs with indoles (**5**), benzofurans (**6**), imidazo[1,2-*a*]pyridines (**7**), and pyrazolo[1,5-*a*]pyridines (**8**).

Scheme 1 illustrates the synthesis of 2-substituted indole P1′ series. Reaction of methyl 4-formylbenzoate **9** with 2-methylindole **10** using triethylsilane and trifluoroacetic acid in dichloromethane provided the benzyl-substituted indole **11**,[11] which was hydrolyzed to acid **12**. Compound **12** was coupled with amine **13**[9] using BOP reagent to yield amide **14**. Treatment of **14** with hydroxylamine and sodium methoxide in methanol gave **15**, which was deprotected with TFA to yield **16**. Compounds **17**–**19** were synthesized using a similar sequence.

Scheme 2 shows the synthesis of analogs of the benzofuran P1′ series. Ketone **20** was alkylated with methyl 4-bromomethylbenzoate using sodium hydride in THF to provide **21**. Treatment of **21** with boron tribromide in dichloromethane resulted in selective demethylation of the phenyl ether, formation of cyclic hemiketal and dehydration in one pot to give the desired benzofuran **22**. After hydrolysis of the ester, the resultant acid **23** was coupled with amine **24**[8] using BOP reagent followed by hydroxamic acid formation to yield compound **25**. Compounds **26**–**28** were synthesized following a similar sequence.

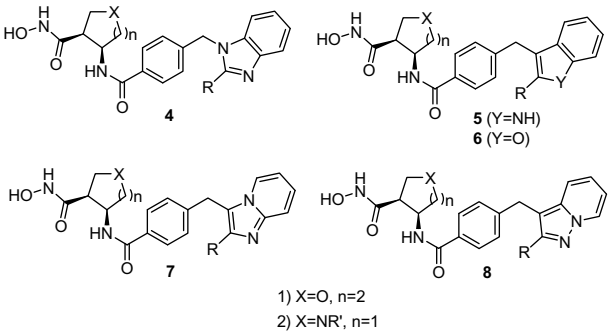
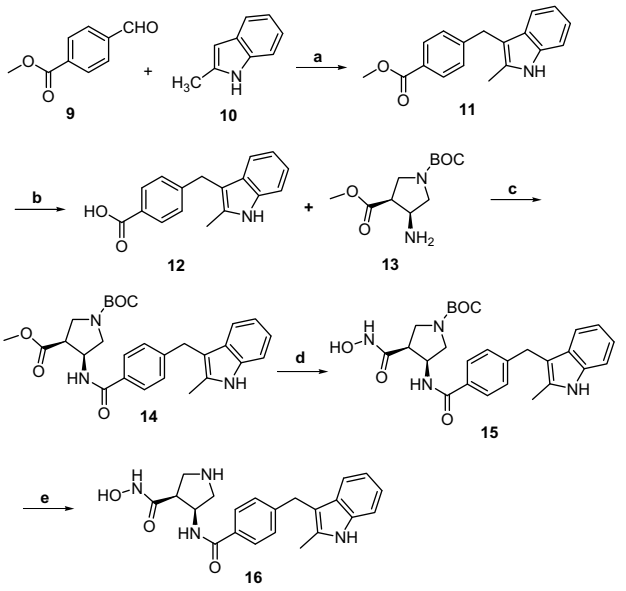
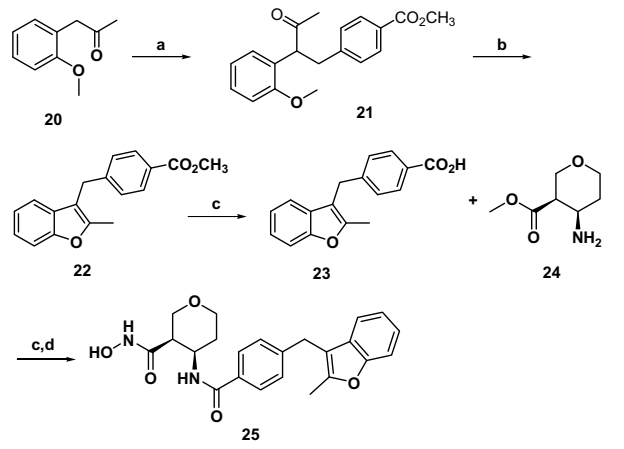


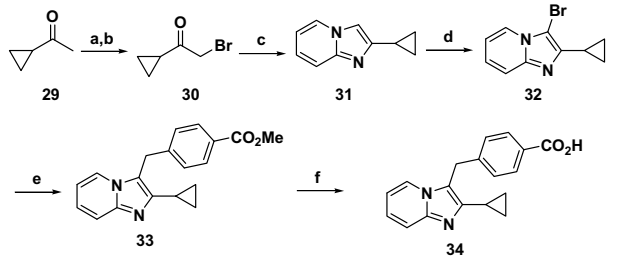
Figure 2. Tetrahydropyran and pyrrolidine-β-aminohydroxamic acid scaffolds with [5,6] bicyclic heterocycle P1′ substituents.



Scheme 1. Reagents: (a) Et_3SiH, TFA, CH_2Cl_2 (78%); (b) LiOH, H_2O, THF (99%); (c) BOP, DIPEA, DMF (87%); (d) NH_2OH, NaOMe, MeOH (50%); (e) TFA, CH_2Cl_2 (100%).



Scheme 2. Reagents: (a) methyl 4-bromomethylbenzoate, NaH, THF (76%); (b) BBr_3, CH_2Cl_2(70%); (c) NaOH, H_2O, MeOH (100%); (d) BOP, DIPEA, DMF (90%); (e) NH_2OH, NaOMe, MeOH (54%).



Scheme 3. Reagents and conditions: (a) LDA, TMSCl, THF (79%); (b) Br_2, CH_2Cl_2 (97%); (c) 2-aminopyridine, EtOH, at reflux (16%); (d) Br_2, CH_2Cl_2 (83%); (e) 4-(methoxycarbonyl)benzylzinc bromide, $Pd(PPh_3)_4$, THF (33%); (f) LiOH, H_2O, THF (73%).

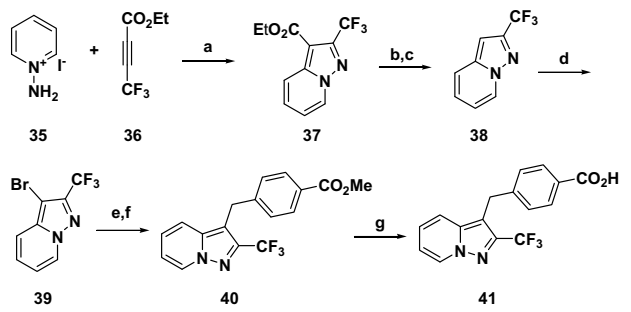
The synthesis of imidazopyridine P1′ acid started from 1-cyclopropylethanone **29** (Scheme 3). Treatment with

LDA followed by chlorotrimethylsilane provided a silyl enol ether, which was reacted with bromine to provide the bromide **30**. Cyclocondensation of **30** with 2-aminopyridine in ethanol at reflux provided the desired imidazopyridine **31** in 16% yield.[12] Subsequent bromination and palladium-catalyzed cross-coupling with 4-(methoxycarbonyl)benzylzinc bromide yielded the ester **33**, which was hydrolyzed to the acid **34**.

The synthesis of pyrazolopyridine P1′ acid is outlined in Scheme 4. Following a procedure reported by Potts, 1-aminopyridinium iodide **35** was reacted with ethyl 1,1,1-trifluoro-2-butynoate **36** via [3 + 2] cycloaddition followed by air oxidation to provide the pyrazolopyridine **37** in 73% yield.[13] Saponification and decarboxylation of **37** provided compound **38**, which was brominated to yield **39**. After treatment with butyllithium, the lithiated pyrazolopyridine intermediate was reacted with methyl 4-formylbenzoate to give an intermediate alcohol that was reduced with triethylsilane and trifluoroacetic acid to provide **40**. Hydrolysis with NaOH completed the synthesis of acid **41**. Following conditions shown in Schemes 1 and 2 acids **34** and **41** were coupled with β-aminoacid **24** and converted to the hydroxamic acids. Other analogs containing different substituents at 2-position of the imidazopyridine and the pyrazolopyridine were synthesized following a similar sequence.

Compounds were evaluated in vitro using porcine TACE (pTACE), as a result of its availability and homology to human TACE.[14] Selectivity profile was initially tested against MMP-2, 3, -7, and -12. Cellular suppression of TNF-α was measured using human whole blood (WBA) stimulated with lipopolysaccharide (LPS). Pharmacokinetic studies were conducted using cassette-dosing (n-in-1) administered intravenously and orally to Sprague–Dawley rats.

The 2-methylindole group, when combined with a pyrrolidine core (compound **16**, Table 1), gave very potent activity in pTACE and WBA assays (2.2 and 52 nM, respectively). Compound **16** also had excellent selectivity (600-fold or greater) against MMP-2, -3, and -7. However, its selectivity against MMP-12 was only about 73-fold. Introduction of 2-propynyl group to the pyrrolidine nitrogen (compound **17**) failed to improve the selectivity against MMP-12 and led to further loss of selectivity against MMP-3 and -7, even though pTACE and WBA activities were maintained. Replacing the pyrrolidine with a tetrahydropyran (**18**) resulted in a slightly weaker inhibitor compared to compound **16** (6 vs 2.2 nM). Changing the 2-indole substituent from methyl to ethyl improved pTACE potency 6-fold (**18** vs **19**), however, the selectivity against MMP-12 was still not satisfactory. The 2-methylbenzofuran analogs (**25** and **26**), while maintaining good pTACE and WBA potency, resulted in reduced selectivity against MMP-12. The bulky 2-isopropylbenzofuran derivatives (**27** and **28**) led to further loss of WBA potency and failed to improve selectivity against MMP-12.[15] The potential reasons for the loss in WBA activity for the benzofuran analogs may be related to the increase in lipophilicity leading to poor cell permeability. Recently described scaffolds with increased lipophilicity have demonstrated less favorable potency in the WBA.[8,9] Furthermore, studies on the γ-lactam scaffold (**1**) have revealed that subtle changes to the P1′ group can impact WBA potency.[7a]

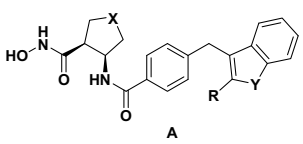


Scheme 4. Reagents and conditions: (a) K_2CO_3, DMF, O_2 (73%); (b) LiOH, H_2O, MeOH (100%); (c) HBr (aq), at reflux (70%); (d) Br_2, EtOH (91%); (e) Methyl 4-formylbenzoate, *n*-BuLi, THF, −78 °C (49%); (f) Et_3SiH, TFA, CH_2Cl_2 (91%); (g) NaOH, H_2O, MeOH (100%).

Next, we turned our attention to imidazo[1,2-*a*]pyridine and pyrazolo[1,5-*a*]pyridine P1′ groups. From this point on, SAR study was carried out using the tetrahydropyran scaffold because it was found to have more desirable oral absorption than the pyrrolidine scaffold. 2-Methylimidazo[1,2-*a*]pyridin-3-yl analog **42** displayed potent activity in pTACE (1 nM) and WBA (122 nM). Furthermore, it had considerably improved MMP selectivity compared to the indole analog (**18**) and benzofuran analog (**25**). In fact, it was greater than 1000-fold selective against all four MMPs. Replacing the 2-methyl substituent with isopropyl and cyclopropyl (**43** and **44**) resulted in two compounds with an almost identical profile. Both increased WBA potency to 74 nM and MMP-12 potency 2-fold. Interestingly, the trifluoromethyl analog **45** was most selective against MMP-12 (>2000-fold), even though its potency in WBA dropped by 2-fold compared to **42**. Unfortunately, these imidazo[1,2-*a*]pyridine analogs suffered from poor oral bioavailability in rat. For example, compound **45** had oral bioavailability of 10% in a rat n-in-1 PK study. The low oral bioavailability observed for **45** may, in part, be attributed to poor absorption. In vitro screening parameters predictive of oral absorption (Caco-2) for **45** suggested that this may have adversely impacted oral PK (P_{app} (A to B) = 0.05×10^{-6} cm/s).

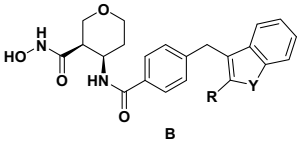
For the pyrazolo[1,5-*a*]pyridine P1′ analogs, 2-ethyl and isopropyl derivatives (**46** and **47**) gave poor selectivity against MMP-12 (K_i of 327 and 119 nM, respectively). The *t*-butyl analog **48** attenuated the MMP-12 activity to 726 nM. Finally, incorporation of a 2-trifluoromethyl group (**49**) totally abolished MMP-12 activity. When **49** was tested in a panel of 11 MMPs, it was inactive in all of the assays (Table 2). Compound **49** also maintained good potency in the LPS–TNF-α whole blood assay (171 nM, Table 1).

Table 1. In vitro profiles of the inhibitors with new heterocyclic P1′ moieties



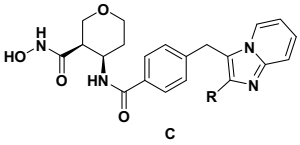
A

Compounds **16, 17, 26, 27**



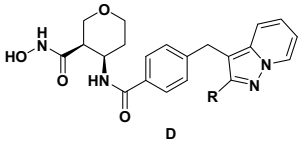
B

Compounds **18, 19, 25, 28**



C

Compounds **42-45**



D

Compounds **46-49**

Compound	Scaffold	X	Y	R	pTACE[a] IC_{50} (nM)	WBA[b] IC_{50} (nM)	MMP-2 K_i (nM)	MMP-3 K_i (nM)	MMP-7 K_i (nM)	MMP-12 K_i (nM)
16	A	NH	NH	Me	2.2	52	>3333	2970	1336	160
17	A	$NCH_2C{\equiv}CH$	NH	Me	1.0	76	2933	852	311	70
18	B	—	NH	Me	6	164	>3333	3690	1527	65
19	B	—	NH	Et	1.0	112	3300	3060	2992	107
25	B	—	O	Me	1.6	168	>3333	1123	3311	53
26	A	NH	O	Me	1.0	220	1367	627	1145	43
27	A	NH	O	*i*-Pr	1.8	1230	>3333	2719	>6368	85
28	B	—	O	*i*-Pr	2.1	626	>3333	766	811	10
42	C	—	—	Me	1.0	122	>3333	>4501	>6368	1021
43	C	—	—	*i*-Pr	3.2	74	>3333	>4501	>6368	580
44	C	—	—	Cyclopropyl	1.0	74	>3333	>4501	>6368	540
45	C	—	—	CF_3	1.5	260	>3333	>4501	>6368	3245
46	D	—	—	Et	2.0	65	>3333	3306	>6368	327
47	D	—	—	*i*-Pr	1.7	108	>3333	3305	2647	119
48	D	—	—	t-Bu	2.8	151	>3333	>4501	>6368	726
49	D	—	—	CF_3	1.0	171	>3333	>4501	>6368	>6023

[a] pTACE IC_{50} and MMP K_i values are average of 2–6 determinations.
[b] Inhibition of TNF-α release in WBA was determined using three donors.

Table 2. Selectivity profile of inhibitor **49**

Enzyme	K_i (nM)
pTACE (IC_{50})	1.0
MMP-1	>4946
MMP-2	>3333
MMP-3	>4501
MMP-7	>6368
MMP-8	>3058
MMP-9	>2128
MMP-10	>5346
MMP-12	>6023
MMP-13	>5025
MMP-14	>5290
MMP-15	>7088

Table 3. Rat n-in-1 pharmacokinetic parameters[a]

	PK parameters	**46**	**48**	**49**
Iv	Dose (mg/kg)	1.0	1.0	1.0
	$t_{1/2}$ (h)	14	0.7	3.4
	Cl (L/h/kg)	0.3	0.7	0.6
	V_{ss} (L/kg)	0.3	0.3	1.0
	AUC (nM h)	5559	2981	3584
Po	Dose (mg/kg)	2.5	2.5	2.5
	$t_{1/2}$ (h)	3.2	2.1	1.9
	AUC (nM h)	7228	3570	10,104
	F%	52	48	>90

[a] Determination of three for each dosing group, average value.

In contrast to the Caco-2 result for **45** containing the isomeric imidazopyridine heterocycle, examples **46–49** containing the pyrazolopyridine heterocycle demonstrated Caco-2 values consistent with oral absorption (P_{app} (A to B): **46**, 1.1×10^{-6}, **48**, 4.3×10^{-6} and **49**, 1.8×10^{-6} cm/s). Compounds **46**, **48** and **49** were examined in rat n-in-1 PK studies. The results are shown in Table 3. Generally, these compounds were characterized by low clearance (<1.0 L/h/kg), good oral bioavailability ($F > 48\%$) and high AUC (>3000 nM h, po). Inhibitor **49** stood out as having the best PK profile with oral bioavailability of 90%.

In summary, using β-amino hydroxamic acid scaffolds several heterocycles were discovered to be excellent P1′ substitutents. Among them, the pyrazolopyridine P1′ series showed the best overall profile. In particular, inhibitor **49** proved to be potent for pTACE, selective over a wide panel of MMPs, and potent in the suppression of LPS-induced TNF-α in human whole blood. More importantly, **49** displayed excellent oral bioavailability ($F > 90\%$) in rat n-in-1 PK studies.

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