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Imidazole acetic acid TAFIa inhibitors: SAR studies centered around the basic P_1' group

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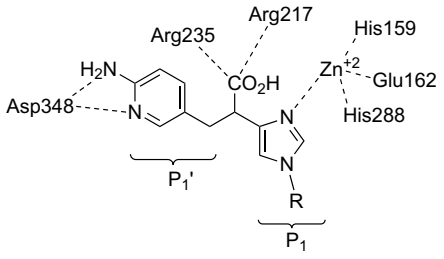
Abstract—Structural modifications of the aminopyridine P_1' group of imidazole acetic acid based TAFIa inhibitors led to the discovery of the aminocyclopentyl analog **28**, a 1 nM TAFIa inhibitor with CLT_{50} functional activity of 14 nM but without selectivity against CPB. While not as active, aminobutyl derivative **27** provided an improved 6.7-fold selectivity for TAFIa versus CPB.

Thrombin-activatable fibrinolysis inhibitor (TAFI) was recently identified[1] as an inhibitor of fibrinolysis.[2] TAFIa is a zinc-containing carboxypeptidase that removes basic C-terminal arginine and lysine residues from peptides and proteins, such as those present on partially degraded fibrin clots. The presence of these residues on fibrin accelerates plasmin generation, and therefore fibrinolysis, by serving as an anchor for the formation of the plasminogen/*t*-PA complex.[3] As a result TAFIa serves as a clot stabilizer and its inhibition should therefore stimulate endogenous fibrinolysis and thereby exert an antithrombotic effect. The activated form (TAFIa), also known as carboxypeptidase U (CPU), carboxypeptidase R (CPR), and plasma carboxypeptidase B, is generated in plasma from its zymogen by limited proteolysis primarily by the thrombin/thrombomodulin complex.[4]

Imidazole acetic acids **1** and **2** represent recent examples[5] of potent TAFIa inhibitors, and the (−) enantiomer of **2** (Fig. 1) was indeed proven to be efficacious in an acute primate model of thrombosis.[5a] Importantly, they are inactive against the critical regulatory carboxypeptidases



R = H: **1**, TAFIa IC_{50} = 0.08 μM, CPB IC_{50} = 0.07 μM
R = $(CH_2)_2$-*i*-Pr: **2**, TAFIa IC_{50} = 0.005 μM, CPB IC_{50} = 0.004 μM

Figure 1. 2-Aminopyridine imidazole acetic acid TAFI inhibitors.

CPM and CPN,[6] and greater than 400-fold selective versus the digestive neutral carboxypeptidase CPA.[7] However, no selectivity versus the digestive basic carboxypeptidase CPB[7,8] was observed, a feature that, hopefully, could be improved on despite the high amino acid sequence homology between CPB and TAFIa.

Based on SAR and on docking studies with a homology model generated[9] from existing X-ray crystallographic structures of CPA and CPB,[10] the probable binding mode of these inhibitors has been determined, and is presented in Figures 1 and 2. In this binding mode, the imidazole moiety of the inhibitor is ligated to the catalytic zinc atom; the carboxylate forms a salt bridge with

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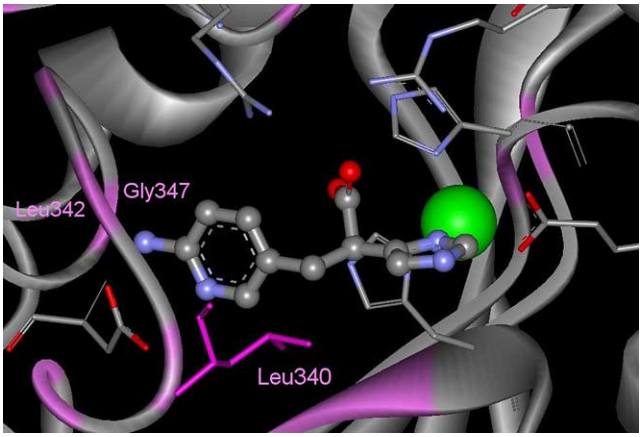


Figure 2. Homology model of TAFIa with inhibitor **1** docked in the active site. Purple residues differ between TAFIa and CPB. Leu340's side chain is shown for clarity.

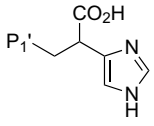
two arginine residues (Arg217 and Arg235); and the 2-aminopyridine moiety fills the S'_1 specificity pocket of the enzyme and forms a salt bridge with aspartic acid 348 at the distal end of the pocket. Alkyl substituents (R) on the imidazole ring occupy the S_1 region of TAFIa and provide potency enhancement but no improvement in selectivity regarding TAFIa versus CPB inhibition.[5a,11]

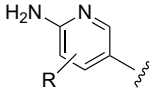
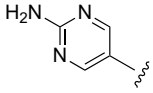
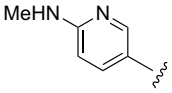
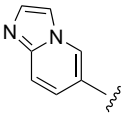
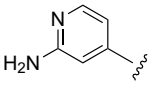
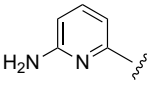
Figure 2 shows residues that differ between human TAFIa and human CPB colored in purple. Specifically highlighted are Leu340, Leu342, and Gly347 that line the P'_1 specificity pocket of TAFIa and exist as Ile, Pro, and Ser, respectively, in CPB.[12] These minor structural dissimilarities between the two carboxypeptidases might be exploited to confer TAFIa specificity versus CPB to the imidazole acetic acid series of inhibitors. Structural modification of the P'_1 aminopyridine group was therefore initiated with the hope to improve both TAFIa inhibitory potency and selectivity versus CPB. Compounds were designed around the imidazole acetic acid template, presenting a variety of basic amino termini in P'_1.

Imidazole acetic acid derivatives presented in this letter were evaluated as racemic mixtures for their inhibitory potency against TAFIa, CPA, CPB, and the regulatory carboxypeptidases CPM and CPN.[13]

Table 1 illustrates modifications of the 2-aminopyridine P'_1 group displayed by inhibitor **1**. Methyl substitution is only tolerated at the 3-position (**3**–**5**). Compound **3**, although almost equipotent to **1**, is less selective versus CPB (0.9-fold and 0.2-fold, respectively). Replacement of the 3-methyl group by electron-withdrawing substituents such as chloro and fluoro (**6**, **7**) or insertion of an

Table 1. 2-Aminopyridine SAR



Compounds	P'_1	R	TAFIa IC_{50} (μM)[a]	CPB IC_{50} (μM)[a]	Selectivity CPB/TAFIa
1		H	0.08	0.07	0.9
3		3-Me	0.1	0.02	0.2
4		4-Me	1.5	—	—
5		6-Me	5.8	—	—
6		3-Cl	5.3	—	—
7		3-F	6.7	—	—
8			67	—	—
9			13	—	—
10			3.3	—	—
11			12	—	—
12			15	—	—

[a] IC_{50} values are the average of at least two determinations, standard error of the mean <10%.

additional nitrogen atom (pyrimidine **8**) are detrimental to potency demonstrating the importance of the Asp348-aminopyridine salt bridge interaction.[14,15] Substitution on either nitrogen atom of the aminopyridine is detrimental to activity (**9**, **10**) presumably due to steric interference with the salt bridge. Isomeric aminopyridine derivatives **11** and **12** are also nearly inactive suggesting optimal basic group presentation to Asp348 for the original aminopyridine **1**.

The data presented in Table 1 demonstrate that the aminopyridine P_1' group cannot easily be improved on, possibly due to the restricted available space in the S_1' pocket. Aminoalkyl P_1' groups therefore emerged as a logical choice since they are sterically smaller and could possibly mimic the lysine residue of the natural substrate. Indeed, the 4-carbon tether of an aminobutyl P_1' group (**14**, Table 2) appeared optimal when compared to aminopropyl and aminopentyl derivatives **13** and **15**. As previously observed, alkylation of the terminal amino group was detrimental to activity (**16** and **17**). Surprisingly, aminobutyl derivative **14** displayed better selectivity for TAFIa versus CPB (6.3-fold) than aminopyridines analogs and it was hoped that it could be further enhanced by substituting the P_1' alkyl linker vide supra. While potency against TAFIa could be further improved in this endeavor (**18** and **19**), selectivity deteriorated as steric bulk increased (5.5-fold and 1.6-fold, respectively), suggesting that the S_1' affinity pocket could be tighter in TAFIa than in CPB.

An alternate strategy to alter potency and selectivity consisted of introducing a ring in the aminoalkyl P_1' side chain, as shown in Figure 3. Incorporation of piperidine, pyrrolidine, and azetidine rings produced analogs of general structure **20**, none of which showed significant activity against TAFIa. Introduction of an exocyclic amino group appeared more promising as illustrated by aminocyclohexyl-methyl analog **22**. Eventually, the installation of an aminocyclopentyl-methyl P_1' group proved very fruitful. The (1*S*,2*R*) isomer **23** was identified as a 100 nM inhibitor of TAFIa although with a modest 1.5-fold selectivity against CPB. All other possible stereoisomers displayed TAFIa $IC_{50} > 4\,\mu M$.

P_1' findings were then applied to the substituted imidazole scaffold illustrated by inhibitor **2**. As expected,[5a]

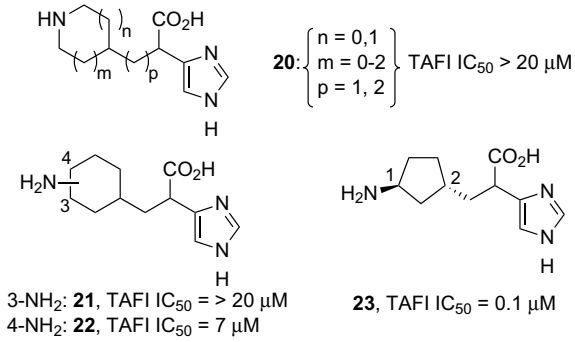
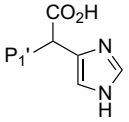


Figure 3. Cyclic aminoalkyl and aminocyclopentyl inhibitors.

addition of a P_1 group to the imidazole moiety (isopentyl or 3,3-dimethylbutyl) resulted in potency enhancement (Table 3). Functional activity (CLT_{50}) was also assessed for inhibitors **24**–**28** using an in vitro clot lysis assay.[5a] CLT_{50} values represent the inhibitor concentration providing 50% of the maximal acceleration of clot lysis in pooled human plasma, triggered by thrombin, $CaCl_2$, and *t*-PA, and detected by turbidity changes. While each inhibitor in Table 3 displays a CLT_{50} in the 10–200 nM range, analogs **27** and **28** stand out. Aminobutyl derivative **27**, although functionally 2-fold less active than aminopyridine analog **2**, is more selective for TAFIa versus CPB (6.7-fold and 0.8-fold, respectively). Aminocyclopentyl derivative **28** was identified as one of the most intrinsically and especially functionally active TAFIa inhibitor ($IC_{50} = 1$ nM, $CLT_{50} = 14$ nM), unfortunately it lacked any selectivity against CPB.

Imidazole acetic acid derivatives presented in this letter were prepared according to Scheme 1. Substituted aminopyridine alkylating agents of type **30** were synthesized from halides **29** via carbonylation, reduction, and bromination. Alkylation of malonate derivative **31**[5a] provides intermediates **32**. Hydrolysis/decarboxylation with 6 N HCl provides imidazole acetic acid derivatives **1**–**7** while selective tosyl removal and imidazole alkylation leads to analogs **2** and **24**–**26**. Inhibitors **8**–**12** were synthesized from the corresponding alkylating agents, prepared using known procedures.[16] Aminoalkyl derivatives **13**–**23**, **27**, and **28** were derived from the corresponding protected aminoalkyl P_1' iodides and **31**, in the same manner as described above. The necessary iodides

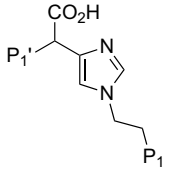
Table 2. Aminoalkyl SAR



Compounds	P_1'	TAFIa IC_{50} (μM)[a]	CPB IC_{50} (μM)[a]	Selectivity CPB/TAFIa
13	H_2N–$(CH_2)_3$	6.8	—	—
14	H_2N–$(CH_2)_4$	0.59	3.7	6.3
15	H_2N–$(CH_2)_5$	4.7	—	—
16	MeHN–$(CH_2)_4$	26	—	—
17	Me_2N–$(CH_2)_4$	>50	—	—
18	H_2N–CH_2–CHMe–$(CH_2)_2$	0.27	1.5	5.6
19	H_2N–CH_2–CMe_2–$(CH_2)_2$	0.38	0.6	1.6

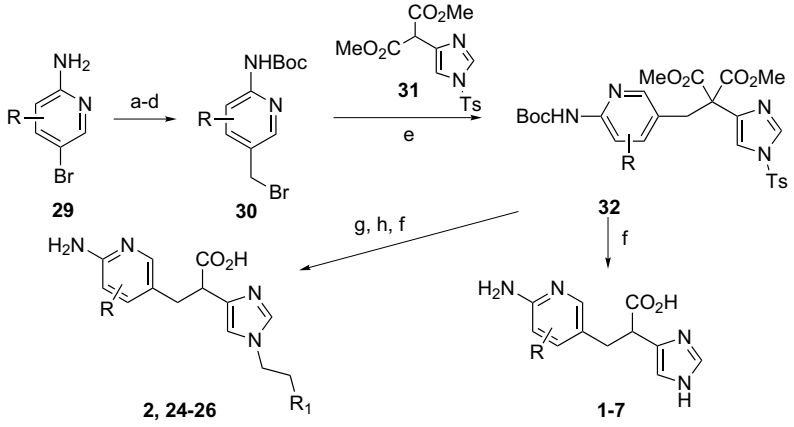
[a] IC_{50} values are the average of at least two determinations, standard error of the mean <10%.

Table 3. P_1–P'_1 combinations



Compounds	P'_1	P_1	TAFIa IC_{50} (μM)[a]	CPB IC_{50} (μM)[a]	Selectivity CPB/TAFIa	CLT_{50} (μM)[b]
1		H	0.08	0.07	0.9	0.970
2		*i*-Pr	0.005	0.004	0.8	0.120
24		*t*-Bu	0.002	0.004	2	0.071
3		H	0.1	0.02	0.2	2.1
25		*i*-Pr	0.023	0.001	0.04	0.170
26		*t*-Bu	0.012	0.001	0.08	0.159
14		H	0.59	3.7	6.3	>20
27		*i*-Pr	0.024	0.160	6.7	0.210
23		H	0.1	0.15	1.5	0.44
28		*t*-Bu	0.001	0.001	1	0.014

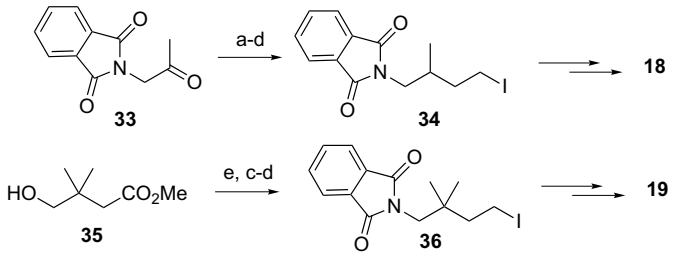
[a] IC_{50} values are the average of at least two determinations, standard error of the mean <10%.
[b] CLT_{50} values represent inhibitor concentration providing 50% acceleration of clot lysis in pooled human plasma, triggered by thrombin, $CaCl_2$, and *t*-PA, and detected by turbidity changes.[5a] Values are the average of at least two determinations, standard error of the mean <10%.



Scheme 1. Reagents and conditions: (a) Boc_2O, DMAP, 60–95%; (b) CO, Pd^0, Et_3N, 50–60%; (c) LAH, 60–85%; (d) Ms_2O, LiBr, or CBr_4, Ph_3P, 50–80%; (e) NaH, **31**, 30–90%;[6] (f) 6 N HCl, 100 °C, 30–95%; (g) cat. MeONa, MeOH, 70–95%; (h) $R_1(CH_2)_2OTf$, 60–85%.

were obtained from readily available protected (phthalimide or Boc) aminoacids or aminoalcohols via reduction and iodination (I_2, Ph_3P, imidazole). Substituted aminobutyl analogs **18** and **19** were prepared according to Scheme 2. Iodide **34** was synthesized from commercially available ketone **33** via Wittig reaction followed by reduction and iodination. Hydroxyester **35**[17] was converted to iodide **36** via Mitsunobu reaction with phthalimide followed by reduction and iodination.

Optimization of the P'_1 group of imidazole acetic acid TAFIa inhibitors led to the discovery of the aminocyclopentyl analog **28**, a 1 nM TAFIa inhibitor with a CLT_{50} functional activity of 14 nM. While not as active as **28**, aminobutyl derivative **27** provided an improved 6.7-fold selectivity for TAFIa versus CPB. The data presented in this letter indicates that the S1′ affinity pockets in TAFIa and in CPB present only subtle structural differences and that additional studies will be required to combine exquisite potency and selectivity.



Scheme 2. Reagents and conditions: (a) Ph_3PCHCO_2Me, 77%; (b) H_2, $Pd(OH)_2/C$, 74%; (c) DIBAL-H, 30–40%; (d) I_2, Ph_3P, imidazole, 60–65%; (e) phthalimide, Ph_3P, DEAD, 24%.

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8. The significance of CPB inhibition in vivo remains to be determined, as CPB is thought to be present only in the gastrointestinal tract.
9. The homology model of TAFIa was created from the crystal structure of CPA (PDB code 2CTC) using MOE software (Chemical Computing Group, Inc., Montreal). Ten intermediate models were created, and the best-scoring model was minimized to a root-mean-squared gradient of 1 kcal/mole Å. The positions of the Zn, Zn-binding residues (His159, Glu162, and His288), and Asp348 were altered to those found in the crystal structure of procarboxypeptidase B (PDB code 1NSA). These residues, along with the residues flanking them, were allowed to relax in the context of the entire protein using the AMBER force field and the GB/SA solvation model as implemented in BatchMin (Schrödinger, Inc., Portland, OR) with the Zn–ligand distances constrained to those found in 1NSA.
10. (a) Aviles, F. X.; Vendrell, J.; Guasch, A.; Coll, M.; Huber, R. *Eur. J. Biochem.* **1993**, *211*, 381–389; (b) Vendrell, J.; Querol, E.; Aviles, F. X. *Biochem. Biophys. Acta* **2000**, *1477*, 284–298; (c) Christianson, D. W.; Lipscomb, W. N. *Acc. Chem. Res.* **1989**, *22*, 62–69.
11. A full account of our studies regarding SAR studies in P_1 is in preparation. Stauffer, S. R. et al.
12. Whereas Leu340 directs its side chain toward the inside of the S_1' specificity pocket, Pro342's side chain is directed away from the active site, and access to Gly347 is hindered by Asp348 and Asn234. Leu340/Ile340 is therefore expected to have a more direct influence on compounds' selectivity.
13. All compounds presented in this letter were found to be greater than 100-fold selective versus CPA, CPM, and CPN.
14. Calculated (Chemdraw) pK_a's for protonated 2-amino-3-R-5-methyl-pyridines and 2-amino-5-methyl-pyrimidine corresponding to compounds **1**, **3**, **6**, **7**, **8** are 7.0, 7.3, 4.3, 4.1, and 4.3, respectively.
15. Direct replacement of the amino group with hydrogen or chloro on inhibitor **1** results in a 140-and 300-fold loss in potency, respectively.
16. (a) For **8** see: Linschoten, M. et al. *WO00/66557*; (b) Compound **9** was derived from methylation of the NHBoc intermediate; (c) For **10** see: Sanderson, P. E.; Naylor-Olsen, A. M. *US6093717*; (d) For **11** and **12** see: Askew, B. C. et al. *US5852045*.
17. See: Belley, M. L. et al. *US5428033*.