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Identification of orally bioavailable, non-amidine inhibitors of Urokinase Plasminogen Activator (uPA)

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ABSTRACT

In this Letter we report the synthesis and evaluation of a series of non-amidine inhibitors of Urokinase Plasminogen Activator (uPA). Starting from compound **1**, a significant change provided compounds in which the amidine, binding in the S1 pocket, was replaced with a primary amine. Further modifications led to the identification of potent, selective, and orally bioavailable uPA inhibitors.

Urokinase Plasminogen Activator (uPA) is a serine protease that is involved in various biological processes and functions as a key initiator in extracellular proteolytic cascades.[1] Urokinase activates plasminogen to plasmin which in turn initiates the activation of many matrix metalloproteases (MMPs). Plasmin and activated MMPs are capable of degrading collagen, elastin and many other extracellular matrix (ECM) and basement membrane (BM) proteins which can lead to an increase in tissue destruction.[2] Recent studies have associated the tissue remodeling activity of uPA, plasmin, and MMPs in several diseases and disorders, including aneurysm formation and other acute coronary syndromes. Additionally, stroke, multiple sclerosis, rheumatoid arthritis, cancer, and AIDS have also been implicated.[3] Therefore, inhibition of uPA may be useful in the treatment of diseases and disorders mediated by the degradation of ECM and BM proteins.

In light of the potential benefits of uPA inhibition, many articles have described the discovery of uPA inhibitors. However, almost all of the non-peptidic small molecule inhibitors described to date feature either a guanidine or amidine forming a key interaction with the Asp189 carboxylate in the S1 pocket of the active site of Urokinase.[4] So far, only one uPA inhibitor has been described that does not use an amidine or guanidine to make this key interaction. A primary amine, discovered by X-ray fragment screening at Astex, has been shown to be able to fit in the S1 pocket.[5]

Our starting point for development of an orally bioavailable uPA inhibitor began with amidine **1** (Fig. 1), a proprietary molecule derived from our earlier work on serine protease inhibitors and identified through a focused screening effort. Compound **1** is potent against uPA (IC_{50} = 0.098 μM) and shows some selectivity against two related trypsin family serine proteases, tPA and plasmin (IC_{50} = 3.9 and 2.1 μM, respectively). However, compounds of this class were found to generally have low oral bioavailability, a result mainly attributed to the presence of an amidine moiety.

Following analysis of the X-ray crystal structure of **1** bound to the protease domain of uPA (not shown), we found that the amidine does indeed form a salt bridge to Asp189 in the S1 pocket of uPA. Though we needed to keep this important interaction, early

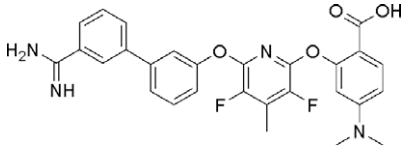

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Figure 1. Focused screening lead.

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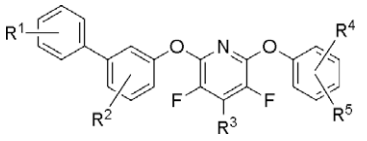
optimization efforts focused on significantly modifying the amidine. Most modifications were unsuccessful, but replacement of the amidine with a benzyl amine provided **2** (IC_{50} = 0.10 μM) (Table 1), a potent compound that showed an improvement in selectivity against tPA and plasmin (IC_{50} = 18 and 6.5 μM, respectively). Additionally, this change led to a compound that showed some oral bioavailability (%*F* = 9) but with low exposure levels after oral administration to rats (Table 2).

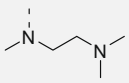
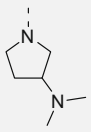
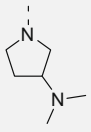
An X-ray structure of **2** bound to uPA (not shown) provided evidence that the benzyl amine had indeed replaced the amidine binding in the S1 pocket. Modifications of the primary amine by substitution of the amine with one or two methyl groups (**3** and **4**, respectively), or moving the benzyl amine to the 4-position (**5**) of the phenyl ring provided only inactive compounds (Table 1). In general, modification of this portion of the molecule led to inactive compounds, highlighting just how specific the S1 pocket is.

Table 2
PK results for selected compounds

Compd	PK data				
	C_{max} (ng/mL)	$T_{1/2}$ (h)	Cl (mL/min/kg)	L_d (L/kg)	%*F*
2	33	0.5	26	1.15	9
11	0	4.6	5	1	0
20	220	0.5	54	0.7	37
26	59	0.26	257	4.6	18

Table 1
Ring variations



Compd	R^1	R^2	R^3	R^4	R^5	uPA IC_{50}[a] (μM)	tPA IC_{50}[a] (μM)	Plasmin IC_{50}[a] (μM)
1	3-C(NH)NH_2	H	Me	2-CO_2H	5-NMe_2	0.098	3.9	2.1
2	3-CH_2NH_2	H	H	2-CO_2H	5-NMe_2	0.10	18	6.5
3	3-CH_2NHMe	H	H	2-CO_2H	5-NMe_2	>30	NA	NA
4	3-CH_2NMe_2	H	H	2-CO_2H	5-NMe_2	>30	NA	NA
5	4-CH_2NH_2	H	H	2-CO_2H	5-NMe_2	>30	NA	NA
6	3-CH_2NH_2	H	NHMe	2-CO_2H	5-NMe_2	0.13	16	3.6
7	3-CH_2NH_2	H	NMe_2	2-CO_2H	5-NMe_2	0.53	>30	2.9
8	3-CH_2NH_2	H	Morpholine	2-CO_2H	5-NMe_2	0.90	10	2.1
9	3-CH_2NH_2	H	Piperazine	2-CO_2H	5-NMe_2	0.065	11	4.4
10	3-CH_2NH_2	H		2-CO_2H	5-NMe_2	0.10	28	6.3
11	3-CH_2NH_2	H		2-CO_2H	5-NMe_2	0.025	5.2	1.9
12	3-CH_2NH_2	H	H	2-CO_2H	H	0.84	>30	21
13	3-CH_2NH_2	H	H	3-CO_2H	H	20	NA	NA
14	3-CH_2NH_2	H	H	4-CO_2H	H	>30	NA	NA
15	3-CH_2NH_2	H	H	2-CH_2CO_2H	H	8.1	NA	NA
16	3-CH_2NH_2	H	H	2-$(CH_2)_2CO_2H$	H	>30	NA	NA
17	3-CH_2NH_2	H	H	2-tetrazole	5-Me	13	NA	NA
18	3-CH_2NH_2	H	H	2-CO_2H	3-Me	26	NA	NA
19	3-CH_2NH_2	H	H	2-CO_2H	4-Me	3.5	NA	NA
20	3-CH_2NH_2	H	H	2-CO_2H	5-Me	0.24	22	5.0
21	3-CH_2NH_2	H	H	2-CO_2H	6-Me	0.54	NA	8.6
22	3-CH_2NH_2	H	H	2-CO_2H	5-*i*Pr	0.68	NA	2.8
23	3-CH_2NH_2	H	H	2-CO_2H	5-OMe	0.22	>30	9.4
24	3-CH_2NH_2	H	H	2-CO_2H	5-NO_2	1.3	NA	NA
25	3-CH_2NH_2	2-NH_2	H	2-CO_2H	5-Me	0.089	15	11
26	3-CH_2NH_2	3-NH_2	H	2-CO_2H	5-Me	0.039	>30	5.0
27	3-CH_2NH_2	4-NH_2	H	2-CO_2H	5-Me	>30	NA	NA
28	3-CH_2NH_2	3-CH_2NH_2	H	2-CO_2H	5-Me	2.7	NA	2.7 ± 0.3
29	3-CH_2NH_2	3-OH	H	2-CO_2H	5-Me	0.023	14	5.5
30	3-CH_2NH_2	3-NH_2		2-CO_2H	5-Me	0.015	8.5	3.0

[a] Values are the mean of ⩾2 experiments. For assay descriptions see Ref. 6. NA = not available.

Fortunately, other parts of the molecule were more amenable to exploration. The 4-position on the pyridine ring (R^3) was modified, beginning with *N*-methyl substituted **6**. In this case, a compound equipotent with **2** was realized. Increasing the size of the R^3 substituent caused a decline in potency as seen in dimethylamine **7** (IC_{50} = 0.53 μM) and morpholine analog **8** (IC_{50} = 0.90 μM). However, when a piperazine was placed in this position, the potency was reclaimed as seen in **9** (IC_{50} = 0.065 μM). The cyclic nature of the amine was not specifically required as linear analog **10** was equipotent with **2**. The racemic dimethylamino pyrrolidine **11** was the most potent analog in this series (IC_{50} = 0.025 μM) and showed selectivity against tPA and plasmin (IC_{50} = 5.2 and 1.9 μM, respectively). Unfortunately, compound **11** had a complete lack of oral bioavailability (Table 2), a result directly attributed to incorporating more charge in the molecule.

Throughout our optimization process, X-ray crystal structures were a valuable tool and were used frequently in analog design. Figure 2 shows one such structure, the complex formed between **11** and human uPA.[7] The free amine binds in the bottom of the S1 pocket and forms a salt bridge to Asp189 (2.9 Å). This amine also has a hydrogen bond to the carbonyls of Ser190 (2.8 Å) and Gly218 (3.0 Å). The carboxylic acid forms several hydrogen bonds. One oxygen interacts with the side chains of Ser195 (2.7 Å) and His57 (2.8 Å) of the catalytic triad. The other oxygen forms a hydrogen bond with the backbone amide nitrogen of Gly193 (2.8 Å). These two key interactions formed the main contact points between our inhibitors and the enzyme. An additional interaction is observed between the pyrrolidine and the enzyme. The dimethylamino pyrrolidine comes in close contact with the loop from residues 96–98. This loop contains two additional residues that block the S4 pocket found in other proteases such as tPA and plasmin. The dimethylamine may pick up some positive electrostatic interactions between the basic nitrogen and the carbonyls of Leu97 (4.0 Å) and Ala98 (4.6 Å) that account for the increase in potency seen with this analog.

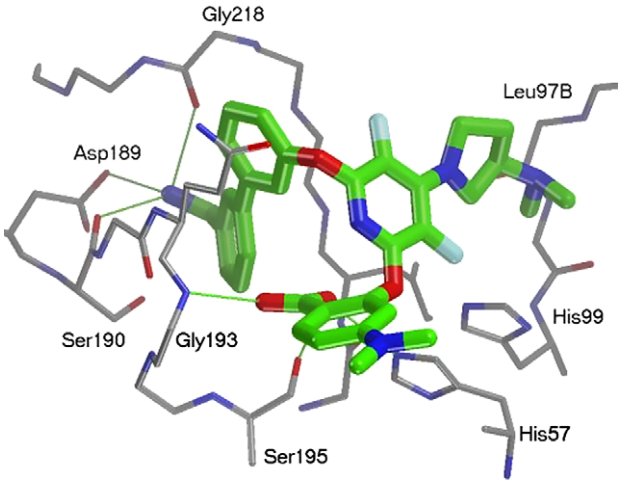


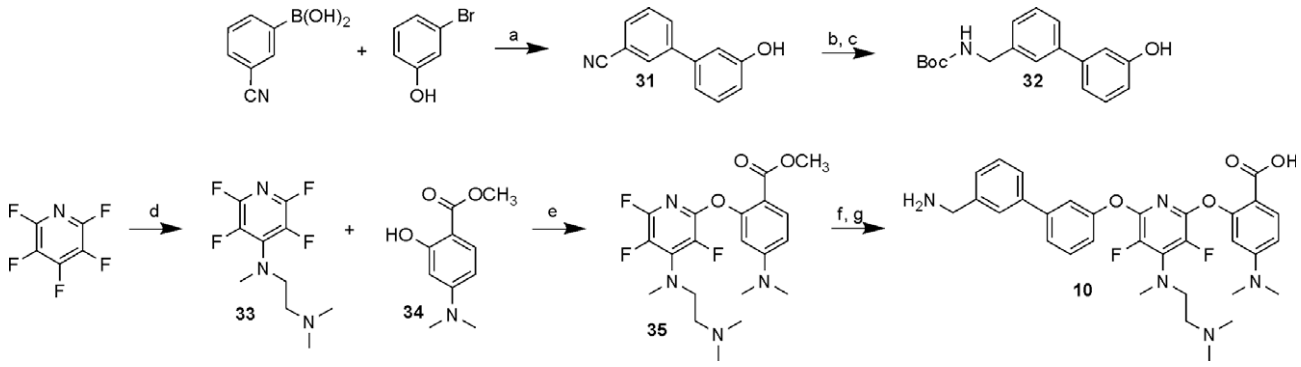
Figure 2. X-ray crystal structure of compound **11** bound to human uPA.

Since compound **2** is zwitterionic, its overall PK was going to be difficult to dramatically improve. As the amine is crucial, we focused on modifying the carboxylic acid group. For ease of synthesis, compound **12** was made as a standard to compare the following analogs. Attempts to either move the acid to other positions on the phenyl ring (**13**, **14**) or change the distance between the acid and aromatic ring (**15**, **16**) reduced the potency. Additionally, the acid isostere **17** was synthesized, but only a weakly active compound resulted from this effort. In general, modification of the carboxylic acid was not tolerated.

Other modifications to the phenyl ring containing the carboxylic acid proved more fruitful. A methyl scan of the ring (**18**–**21**) revealed that the 5-position was favored. Compound **20** (IC_{50} = 0.24 μM) was only twofold less potent than **2** and had similar selectivity against tPA and plasmin (**(**IC_{50} = 22.1 and 5.0 μM, respectively). This was an interesting result especially when compared to isopropyl **22** (IC_{50} = 0.68 μM), a compound that is sixfold less potent than **2**. Another small surprise was observed when a PK study was performed on **20**. This compound was found to have an improved %*F* and C_{max} when compared to **2**, a result we attributed to the reduction of overall charge of the molecule. Further work on this ring showed electron donating groups such as methoxy **23** (IC_{50} = 0.22 μM) provided compounds with greater potency than electron withdrawing groups such as nitro **24** (IC_{50} = 1.3 μM).

With the knowledge gained so far regarding our template, we explored substitutions on R^2. From our X-ray crystal structure studies, we postulated that these substituents should access the S1B subsite. This S1B subsite was so named by Nienaber et al. and exploited in improving their uPA inhibitors.[8] An amino scan revealed that the 2-position **25** (IC_{50} = 0.089 μM) improved the potency some and the 3-position **26** (IC_{50} = 0.039 μM) provided nearly a sixfold improvement compared to **20**. However, 4-position **27** was inactive, probably due to a change in the orientation of the first two aromatic rings with respect to each other. Compound **26** was profiled further and showed an increase in selectivity (IC_{50} >30 and =5.0 μM against tPA and plasmin), some oral bioavailability, but an extremely high clearance.

In general, we found that at R^2 a variety of substituents were tolerated and some provided an improvement in potency. However, R^2 is a sensitive position as exemplified by methylene amine



Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, IPA, 2 N Na_2CO_3(aq), 80 °C, 71%; (b) RaNi, IPA, MeOH, H_2, 94%; (c) Boc_2O, NaOH(aq), dioxane, 95%; (d) *N,N,N′*-trimethylethylenediamine, Et_3N, CH_2Cl_2, 0 °C, 100%; (e) Cs_2CO_3, DMF, 50 °C, 91%; (f) **32**, Cs_2CO_3, DMSO, 80 °C, 73%; (g) (i) LiOH, H_2O, MeOH, 50 °C, 24 h; (ii) TFA, CH_2Cl_2, 57%.

compound **28** losing 10-fold in activity. Interestingly, the hydroxyl **29** (IC_{50} = 0.023 μM) regains the potency seen in the amino series. Combining many of the best groups from each ring provided the potent racemic compound **30** (IC_{50} = 0.015 μM). This analog showed that the effects at each site were additive, which could be useful in further lead optimization.

The four ringed structure of compound **1** makes a modular modification strategy ideal for SAR exploration. Numerous analogs were synthesized in a rapid and convergent fashion simply by varying the order of synthetic steps for incorporating an appropriately functionalized ring. The synthesis of compound **10** (Scheme 1) is representative of the methodology used to prepare the compounds described in this Letter.[9] A Suzuki coupling of commercially available 3-cyano phenyl boronic acid and 3-bromophenol provided nitrile **31**. Following reduction of the nitrile with Raney Nickel, protection of the free amine using Boc_2O provided left side biaryl **32**. The right side biaryl ether was synthesized using two regioselective S_NAr reactions. Reaction of pentafluoropyridine with *N*,*N*,*N*′-trimethylethylene-diamine at lower temperature cleanly provided the 4-position substituted pyridine **33**. Upon treatment of **33** with salicylate **34** using cesium carbonate in DMF and mild heat, biaryl ether **35** was realized. The third S_NAr reaction, between **35** and **32**, required cesium carbonate in DMSO and moderate heat to give the fully assembled ring system. Subsequent saponification and Boc removal provided compound **10**. As a side note, the saponification of the methyl ester in these compounds proved to be time consuming and use of the *tert*-butyl ester in later work was advantageous.

In conclusion, we have described the discovery of a series of non-amidine, multi-ring compounds that function as potent and selective uPA inhibitors. Using a modular approach in analog design and synthesis allowed the modification of molecular properties based on specific portions of the molecule. Further work on this template will be reported in the near future.

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9. All final compounds were characterized by NMR, HPLC, MS, and EA. Additional synthetic details will be provided in future publications and patents.