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Selective Urokinase-Type Plasminogen Activator (uPA) Inhibitors. Part 1: 2-Pyridinylguanidines

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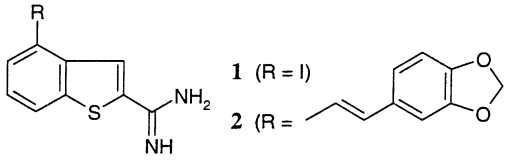
Abstract—The identification of 2-pyridinylguanidines (e.g., **27** and **28**) as selective inhibitors of urokinase-type plasminogen activator (uPA) is described. The X-ray crystal structure of **27** has been determined, and modelling has been used to predict binding in the enzyme active site.

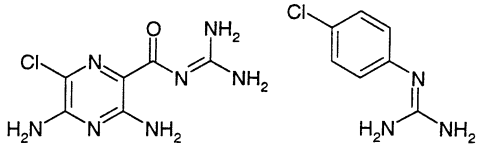
Introduction

Urokinase-type plasminogen activator (uPA) is a trypsin-like serine protease which converts the zymogen plasminogen into its active form plasmin. Studies have indicated that because of its ability to degrade extracellular matrix, either directly or via plasmin formation, uPA is a key mediator of cellular invasion in a range of processes including tumour growth and metastasis, angiogenesis and tissue remodelling.[1,2] Evidence has also been obtained to suggest that uPA, or plasmin produced by its action, may play a role in preventing healing of chronic wounds.[3–7] Consequently, a selective inhibitor for uPA could have therapeutic value in cancer and wound healing.[1–7]

Several related enzymes such as tissue plasminogen activator (tPA) also act via activation of plasminogen to plasmin, and play a key role in the fibrinolytic cascade.[7,8] Therefore, it is desirable that a uPA inhibitor has adequate potency and selectivity for uPA relative to both tPA and plasmin to avoid the possiblity of antifibrinolytic side effects.

Several small molecule inhibitors of uPA are known. For example, aromatic and heteroaromatic amidine derivatives have been reported with moderate potency, but low selectivity over related enzymes.[9–11] Greater selectivity relative to tPA and plasmin has been reported for a series of benzo[*b*]thiophene-2-carboxamidines such as **1**, and the more potent analogue **2**.[12,13] Guanidine derivatives are also reported to have some degree of selectivity including amiloride (**3**)[14] and a series of phenylguanidine derivatives as exemplified by **4**.[15] Since the current work was carried out, a number of templates based upon amidines and guanidines have been reported.[16–24]





3 **4**

In attempting to develop a novel class of uPA inhibitor, we focused upon systems which would bind strongly at the key S_1 binding site and demonstrate intrinsic selectivity over related enzymes. The X-ray crystal structures of **1**, **3** and phenylguanidine bound to re-engineered human uPA have been reported recently, and the results confirm that all three inhibitors bind in the S_1 site of the enzyme.[25] Knowledge of the preference for Arg at S_1 enabled us to limit our attentions to amidines and guanidines, especially the latter where a greater number of the key H-bonding interactions that bind Arg560 of plasminogen would be available. After screening a number of simple guanidine and amidine derivatives, it

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became apparent that aromatic guanidines were favoured, encouraging us to prepare a series of simple guanidino-heterocycles. This paper describes the discovery of 2-pyridinylguanidines as selective inhibitors of uPA, and fundamental SAR in the series. Future papers in the series will describe our efforts to increase potency by introducing substituents capable of achieving binding interactions outside the S_1 binding site.

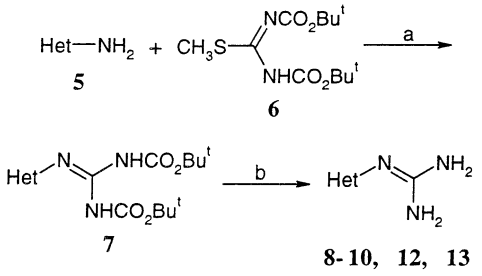
Chemistry

Most of the guanidines described were prepared from the corresponding heterocyclic amines **5** by reaction with *N*,*N*′-bis(*t*-butoxycarbonyl)-*S*-methylisothiourea (**6**) in the presence of mercury(II) chloride, followed by deprotection of the product **7** with either saturated HCl in dichloromethane or with neat trifluoroacetic acid (Scheme 1). In all cases, the starting amines were available commercially or were prepared by literature methods.

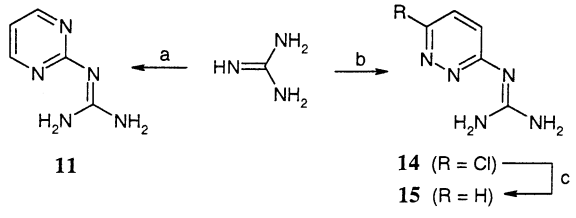
The 2-pyrimidinyl analogue **11** was prepared by the direct reaction of the free base of guanidine with 2-chloropyrimidine. The 3-pyridazinyl analogue **15** was prepared by reaction of guanidine with 3,6-dichloropyridazine followed by dechlorination of the intermediate product **14** by hydrogenolysis (Scheme 2).

Results and Discussion

Compounds were tested for their ability to inhibit uPA, tPA and plasmin as described by Yang et al.[15] The uPA assay was carried out using HWMT human uPA (Calbiochem) and S-2444 (Quadratech) as substrate. The tPA assay was carried out using human tPA (Quadratech), S-2444 as substrate and tPA stimulator (Quadratech). The plasmin assay was carried out using human



Scheme 1. Reagents and conditions: (a) $HgCl_2$, Et_3N (3 equiv), CH_2Cl_2; (b) satd HCl/CH_2Cl_2 or TFA.



Scheme 2. Reagents and conditions: (a) 2-chloropyrimidine, *t*-BuOH; (b) 3,6-dichloropyridazine, DMSO; (c) H_2, Pd/C, EtOH.

plasmin (Quadratech) and Chromozym-PL (Boehringer) as substrate.

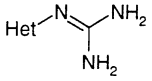
Calculated K_i values for simple heterocyclic guanidine derivatives are listed in Table 1, together with comparative figures for compound **4**.

2-Pyridinylguanidine (**8**) showed encouraging potency against uPA, and excellent selectivity over both tPA and plasmin. However, the 3- and 4-isomers **9** and **10** showed negligible inhibition up to millimolar concentrations, although the latter showed significant activity against plasmin. Introduction of an additional ring nitrogen atom (**11**–**15**) was also detrimental. In view of its potency and selectivity, the 2-pyridinyl analogue **8** was considered a suitable starting point for the design of more potent novel uPA inhibitors, and the effect of simple ring substitution was then explored (Table 2).

Examination of methyl substitution demonstrated that substituents are tolerated at the 3- and 5-positions of the pyridine ring (**16** and **18**), but substitution is highly detrimental at the 6-position (**19**). The 4-methyl isomer **17** was less potent than the parent, but substitution by a phenyl group (**20**) led to a 4-fold increase. Introduction of 5-halo or 3,5-dihalo substituents gave a marked increase in potency, generally without loss of selectivity over tPA and plasmin (**24**–**28**, **30**). Introduction of a 5-CF_3 substituent was unfavourable (cf. **29** with **26** and **28**).

Modelling studies were carried out on the active pyridinylguanidines with the aim of identifying the most suitable position for appending further substitution to increase binding interactions with the enzyme. The X-ray crystal structure of **27** was determined (Fig. 1)[26] and compared with the reported crystal structure of the phenylguanidine derivative **31**.[27] Compared with **31**, compound **27** was found to be more planar (Ar–guanidine torsion angle 5.2° compared with 49°) which we believe to be a result of both the loss of a steric clash on replacing a CH with a N, and formation of an intra-

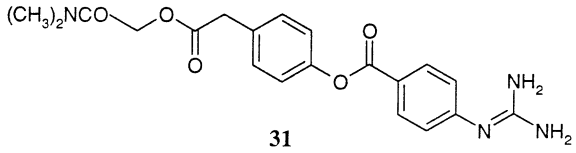
Table 1. Enzyme inhibition data for guanidine-substituted heterocycles



Compd	Het	K_i (μM) or % inhibition		
		uPA	tPA	Plasmin
4		6.70	[a]	[b]
8	2-Pyridinyl	29.5	[b]	[b]
9	3-Pyridinyl	[b]	[b]	[b]
10	4-Pyridinyl	[b]	[b]	16.9
11	2-Pyrimidinyl	292.0	[b]	[b]
12	4-Pyrimidinyl	[b]	[b]	[b]
13	2-Pyrazinyl	[b]	[b]	[b]
15	3-Pyridazinyl	[c]	[c]	[a]

[a] < 50% inhibition at 30 μM.
[b] < 50% inhibition at 1 μM.
[c] < 50% inhibition at 100 μM.

molecular H-bond between the pyridine N atom and a guanidine proton.

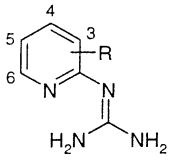


31

Compound **27** was docked and minimised[28] into the human uPA X-ray structure[29] over the GluGlyArg-chloromethyl-ketone, which is covalently bound in this uPA structure (Fig. 2). The available SAR could be rationalised using this model. The pyridinylguanidine system fills the S_1 pocket with the guanidine forming a strong interaction with Asp189 at the bottom of the pocket (Fig. 2a). The 5-substituent must be small to be accommodated as it is directed towards Ser195. Based on the structure of phenylguanidine bound to human uPA, it has recently been proposed that additional binding energy may be obtained by interaction of a small substituent such as the 4-Cl in compound **4** with Ser195.[22] The 4-position in **27** is adjacent to Glu192, but any substituent would lie substantially in solvent and therefore, although a range of substituents would be accommodated, they would not be expected to form extensive interactions with the protein. Substituents at the 3-position are directed along the binding groove towards Arg217, suggesting this as being the best position from which to capitalise upon the available sites of interaction in order to optimise uPA potency. Figure 2b shows more clearly the spatial fit of the molecule into the S_1 pocket, and the 3-substituent pointing along the binding groove.

In summary, we have demonstrated that simple 2-pyridinyl guanidines, especially those with halogen substitution at the 3- and/or 5-positions, are selective inhibitors of uPA. Modeling, using the published X-ray crystal structure of the human enzyme, has been used to predict the likely binding interactions of this class of inhibitor in the S_1 pocket of the enzyme. The results of the modelling suggest that it should be possible to increase potency by introduction of suitable substitution at the 3-position of the pyridine ring to interact in the

Table 2. Enzyme inhibition data for 2-pyridinylguanidine derivatives



Compd	Het	K_i (μM) or % inhibition		
		uPA	tPA	Plasmin
8	H	29.5	[a]	[a]
16	3-CH_3	37.7	[a]	[a]
17	4-CH_3	83.8	[a]	[a]
18	5-CH_3	32.3	[a]	245.0
19	6-CH_3	173.0	[a]	[a]
20	4-C_6H_5	7.10	54% @ 1 mM	[a]
22	4-OCH_3	53.3	[a]	[a]
21	3-OH	147.0	[a]	[a]
23	3-OCH_3	62.0	[a]	232.5
24	5-Cl	10.0	[a]	[a]
25	5-Br	3.13	[a]	[a]
26	3,5-diCl	5.47	[a]	[a]
27	3-Br, 5-Cl	4.83	272.0	[a]
28	3-Cl, 5-Br	2.90	[a]	[a]
29	3-Cl, 5-CF_3	40.7	[a]	[a]
30	3,5-diCl-4-Me	8.70	[a]	176.5

[a] < 50% inhibition at 1 mM.

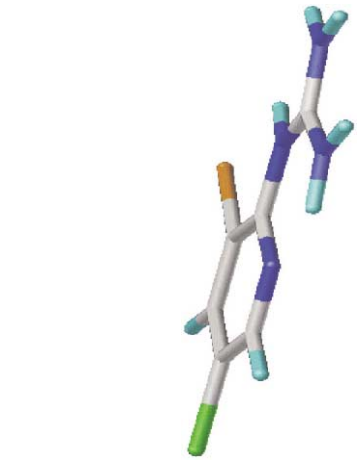
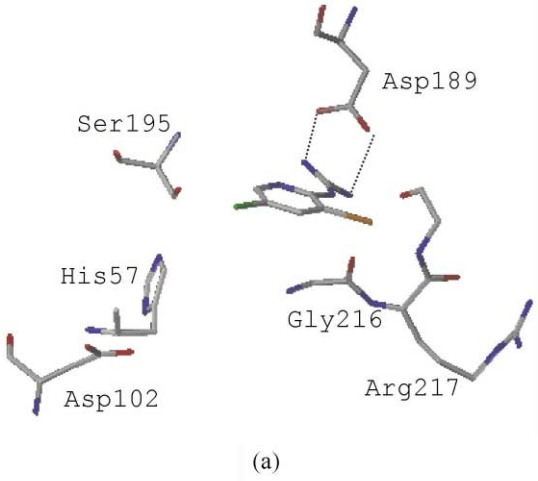
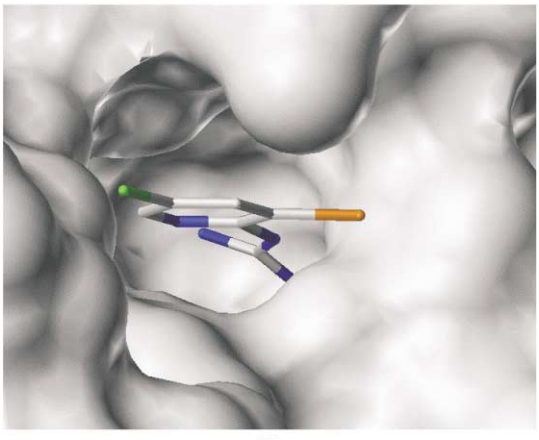


Figure 1. X-ray structure of **27**.



(a)



(b)

Figure 2. Compound **27** docked into active site of uPA.

binding groove of the enzyme. The synthesis and properties of such compounds are described in the following paper.

Acknowledgements

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