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Selective Urokinase-Type Plasminogen Activator (uPA) Inhibitors. Part 2: (3-Substituted-5-halo-2-pyridinyl)guanidines

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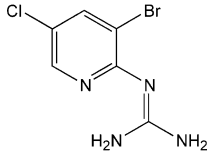
Abstract—Based on previous modeling predictions, a series of (3-substituted-5-chloro-2-pyridinyl)guanidines have been designed with good potency and selectivity for urokinase-type plasminogen activator (uPA). Compound **36** has a K_i of 0.17 μM and greater than 300-fold selectivity with respect to tPA and plasmin.

The previous paper[1] described the identification of 2-pyridinylguanidine derivatives as selective inhibitors of urokinase-type plasminogen activator (uPA). Such compounds are of potential use in disease indications where degradation of extracellular matrix by uPA may be a contributing factor, such as tumor growth and metastasis, angiogenesis and tissue remodeling.[2,3] uPA acts through activation of the zymogen plasminogen to plasmin. Related enzymes such as tissue plasminogen activator (tPA) also act via generation of plasmin from plasminogen,[4,5] so an adequate degree of selectivity over both tPA and plasmin is an important requirement.



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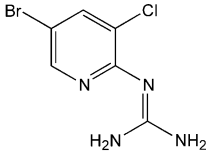

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3,5-Dihalo derivatives such as **1** and **2** were the most potent inhibitors in the previous investigation of simple substitution in the pyridine ring. Modeling studies suggested that the inhibitors bind in the S_1 pocket of the enzyme with the guanidine system interacting with Asp189, and the 5-halo substituent (chlorine or bromine) directed towards the catalytic serine.[1] The modeling results also suggested that introduction of suitable substituents at the 3-position could potentially capitalize on interactions in the binding groove of the enzyme. This paper describes identification of 3-substituted analogues with submicromolar inhibitory activity against uPA and a high degree of selectivity over tPA and plasmin.

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The required guanidines were prepared from a 3-substituted-2-amino-5-chloropyridine derivative, most of which were obtained from the amine **3** using Pd(0)-catalyzed cross-coupling as the key step (Schemes 1 and 2). It was decided to restrict SAR exploration to the 5-chloro series to enable use of chemoselective reactions at the 3 position of the readily-available 2-amino-3-bromo-5-chloropyridine (**3**).[6] Thus, reaction of **3** with *t*-butyl propenoate under Heck conditions gave the propenoic ester **4** which was hydrolysed (TFA), and the resulting acid **5** was converted to the amide derivatives **6**–**9** via activation as the HOBT esters (Scheme 1). Reduction of **4** gave the propanoate derivative **10**.

Arylalkenyl and cycloalkenyl derivatives were prepared similarly except that the Heck reactions were carried out under microwave irradiation (Scheme 2). In the case of **11** (R = 3,4-methylenedioxyphenyl), the product was prepared using a Stille coupling with [(*E*)-2-(1,3-benzodioxol-5-yl)ethenyl]tributyl)stannane.[7] A Heck reaction using phenylethyne was used to prepare the phenylethynyl derivative **12**, and the phenyl derivative **13** was prepared via a Suzuki coupling with benzeneboronic acid.

Starting amines **15**, in which the 3-substituent is an ether, were accessed either by alkylation of 2-amino-5-chloro-3-hydroxypyridine **14**[8] or, in the case of R = methoxyethyl or C_6H_5, by nucleophilic displacement of the bromine in **3** with the corresponding alcohol or phenol under basic conditions (Scheme 3).

Final products (**17**–**35**) were prepared by reaction of the amines with *N*,*N*′-bis(*t*-butoxycarbonyl)-*S*-methylisothiourea (**16**) in the presence of mercury (II) chloride, followed by deprotection with trifluoroacetic acid (Scheme 4). In the case compounds with a *t*-butyl ester

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3-substituent, the final product was the corresponding carboxylic acid. Acid hydrolysis of **35** [R = 2-(3-cyanophenylethenyl)] gave the corresponding carboxylic acid product **36** (Scheme 4).

Compounds were tested for their ability to inhibit uPA, tPA and plasmin as described previously.[1] Results for simple heterocyclic guanidine derivatives, expressed as a calculated K_i, are listed in Table 1, together with comparative figures for **1**.

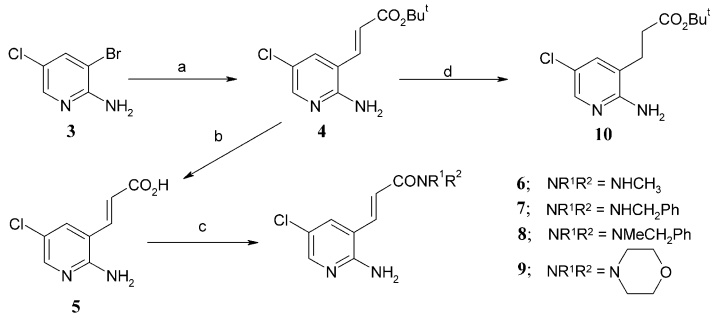
Compared with **1**, introduction of propenoic acid (**17**) or amide 3-substituents (**18**–**21**) gives a small increase in potency, most notably in the case of the benzylamide **20**. Increasing the flexibility of the side chain as in the propanoic acid analogue **22** or the ether analogues **23** and **24** is unfavorable. Introduction of a phenyl substituent (**26**) is detrimental, suggesting an unfavorable steric effect, but activity is restored with the phenyl ether **27**. Further extension of the side chain as in **28** is favorable. Comparison of **28** and **25** clearly demonstrates the desirability of an aryl substituent.

The most potent analogues result from increasing the rigidity of the aryl side chain, for example the arylethenyl derivatives **30** and **34**.

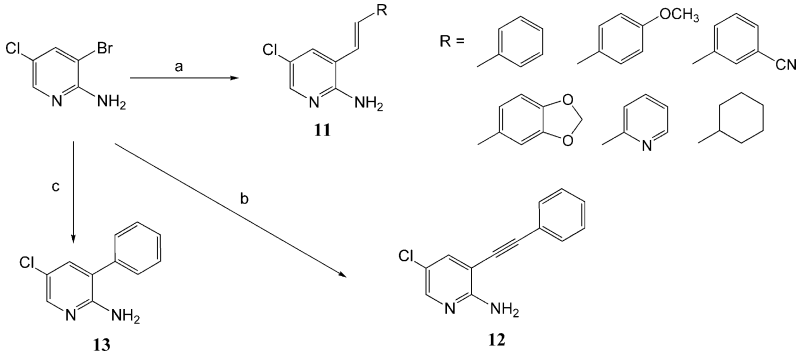
Carboxylic acid derivative **36** was the most active pyridylguanidine that we identified within this series. In the published X-ray crystal structure of uPA complexed with the inhibitor Glu-Gly-Arg chloromethyl ketone, a salt bridge is observable between the terminal Glu carboxylate and Arg217 of the enzyme.[9] It is possible that the higher potency of **36** compared with other compounds in the series results from a combination of hydrophobic binding in the main binding groove of the enyme and formation of an equivalent salt bridge with Arg217.

Some activity against tPA and plasmin is evident with the analogues bearing lipophilic 3-substituents, but in all cases excellent selectivity for uPA is maintained. In the case of **36**, selectivity is at least 300-fold with respect to tPA and plasmin.

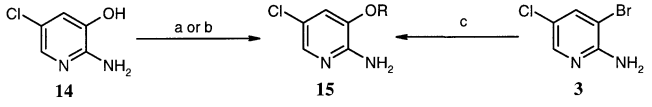
In summary, we have used modeling predictions as a basis for the design of (3-substituted-5-chloro-2-pyridinyl)-guanidines with increased potency against uPA compared with the earlier 3,5-dihalo-substituted analogues. Lipophilic 3-substituents such as arylalkenyl are the most favorable, and the (3-carboxy)phenylethenyl analogue **36** is the most potent inhibitors in this series. Extension of this work to other heterocyclic guanidine derivatives with greater potency will be reported in due course.



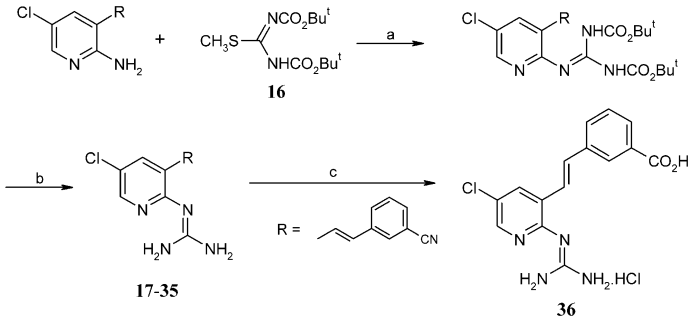
Scheme 1. Reagents and conditions: (a) $H_2C{=}CHCO_2Bu^t$, $Pd(OAc)_2$, P(o-Tol)$_3$, Et_3N, 150 °C (pressure vessel); (b) TFA, 20 °C; (c) R^1R^2NH, HOBT, Hünig's base, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl; (d) $NaBH_4$, EtOH.



Scheme 2. Reagents and conditions: (a) $H_2C{=}CHR$ or $(Bu)_3SnCH{=}CHR$, $Pd(OAc)_2$, P(o-Tol)$_3$, Et_3N, DMF, microwave oven (sealed vessel, 800W, ca. 30 s); (b) $PhC{\equiv}CH$, CuCl, $PdCl_2(PPh_3)_2$, Et_3N, DMF, microwave oven (sealed vessel, 800W, ca. 30 s); (c) $PhB(OH)_2$, $(Ph_3P)_4Pd$, Na_2CO_3, $MeO(CH_2)_2OMe$.

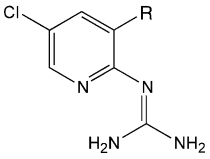


Scheme 3. Reagents and conditions: (a) $BrCH_2CO_2Bu^t$ or $PhCH_2Br$, MeONa, DMSO, 20 °C; (b) $ClCH_2CONHCH_2Ph$, MeONa, DMSO; (c) ROH, KOH, $CuSO_4$ (anhydrous), $MeO(CH_2)_2OMe$, 140–150 °C.



Scheme 4. Reagents and conditions: (a) $HgCl_2$, Et_3N (3 equiv), CH_2Cl_2; (b) satd HCl/CH_2Cl_2 or TFA; (c) concd HCl, AcOH, reflux.

Table 1. Enzyme inhibition data for 2-pyridinylguanidine derivatives



Compd	R	K_i (μM) or % inhibition		
		uPA	tPA	Plasmin
1	Br	4.83	272.0	[a]
17	(*E*)-CH=CHCO$_2$H	1.50	[a]	278.5
18	(*E*)-CH=CHCONHMe	1.28	[a]	[a]
19	(*E*)-CH=CHCO(1-morpholino)	2.10	[a]	[a]
20	(*E*)-CH=CHCONHCH$_2$Ph	0.77	[a]	[a]
21	(*E*)-CH=CHCON(Me)CH$_2$Ph	2.00	[a]	[a]
22	CH$_2$CH$_2$CO$_2$H	9.31	[a]	[a]
23	OCH$_2$CO$_2$H	90.2	[a]	[a]
24	OCH$_2$CONHCH$_2$Ph	16.1	[a]	[a]
25	OCH$_2$CH$_2$OCH$_3$	16.4	[a]	[a]
26	C$_6$H$_5$	166.3	[a]	[a]
27	OC$_6$H$_5$	2.13	342.0	[a]
28	OCH$_2$C$_6$H$_5$	0.92	[a]	[a]
29	C≡CC$_6$H$_5$	0.77	65.9	218.0
30	(*E*)-CH=CHC$_6$H$_5$	0.55	53.8	[a]
31	(*E*)-CH=CHC$_6$H$_{11}$	0.73	71.5	[a]
32	(*E*)-CH=CH(2-pyridinyl)	1.60	258.5	[a]
33	(*E*)-CH=CH(4-C$_6$H$_4$OMe)	0.69	60.1	42.5
34	(*E*)-CH=CH[(3,4-OCH$_2$O)C$_6$H$_4$]	0.49	50% @ 300 μM	[a]
36	(*E*)-CH=CH(3-C$_6$H$_4$CO$_2$H)	0.17	52.0	[b]

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

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References and Notes

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