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Bioorganic & Medicinal Chemistry Letters 14 (2004) 3227–3230
Bioorganic & Medicinal Chemistry Letters

Selective urokinase-type plasminogen activator (uPA) inhibitors. Part 3: 1-Isoquinolinylguanidines

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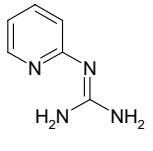
Received 22 December 2003; revised 17 March 2004; accepted 30 March 2004

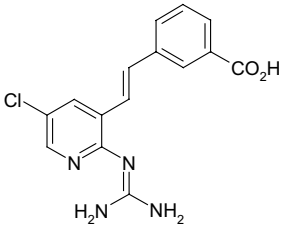
Abstract—A series of 1-isoquinolinylguanidines are shown to be potent inhibitors of uPA with selectivity over tPA and plasmin. Potency is enhanced by the presence of a 4-halo and a 7-aryl substituent, particularly when substituted by a 3-carboxylic acid group. Compound **13j** (UK-356,202) combines excellent potency and selectivity, and has been selected as a candidate for clinical evaluation.

Urokinase-type plasminogen activator (uPA) is a serine protease that has been implicated as a key mediator of cellular invasion and tissue remodeling.[1] An inhibitor of uPA may have a therapeutic role in disease situations where uPA-driven degradation of extracellular matrix, or uPA-dependent cell migration is thought to be important—including tumour growth, metastasis, angiogenesis and chronic wounds.[2–7] The closely related enzyme tPA also acts via activation of plasminogen to plasmin and is a key component of the fibrinolytic cascade.[8,9] Achievement of adequate selectivity over both tPA and plasmin is therefore an important requirement in a therapeutically valuable uPA inhibitor.

We previously reported that 2-pyridinylguanidine (**1**) is a selective inhibitor of uPA, that potency is enhanced by the presence of a 5-halo substituent, and that introduction of various 3-substituents as in **2** gives further increases in potency.[10,11]



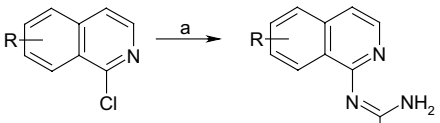
1



2

As part of our programme of exploration of substitution in the pyridine ring, we also investigated benzo-substitution to give quinoline and isoquinoline derivatives. This communication reports our results leading to the identification of 1-isoquinolinylguanidines with enhanced potency and selectivity for uPA.

In most cases, the required isoquinolinylguanidines were prepared by reaction of guanidine with the corresponding 1-chloroisoquinoline (Scheme 1). The 1-chloro intermediates were either known compounds (4-CH_3,[12] 5-OCH_3,[12] 7-OCH_3,[12] 4-Cl,[13] 4-Br,[14] 5-Br[15]) or were prepared from the corresponding isoquinoline by oxidation with either 30% H_2O_2/AcOH or mCPBA/CH_2Cl_2, followed by treatment of the N-oxide with $POCl_3$ (5-C_6H_5,[16] 5-$OCH_2C_6H_5$,[17] 6-Br,[18] 7-$OCH_2C_6H_5$[19]). In the case of 5-bromo-1-chloro- and 7-bromo-1-chloroisoquinolines, a mixture of 5- and 7-bromoisoquinoline[20] was oxidized, the product was treated with $POCl_3$ and the mixture of chloro compounds was separated by



3a-q **4a-q**

Scheme 1. Reagents and conditions: (a) Guanidine HCl, NaH, DMSO, 100 °C.

Keywords: uPA; Urokinase plasminogen activator; UK-356202; 1-Isoquinolinylguanidine.
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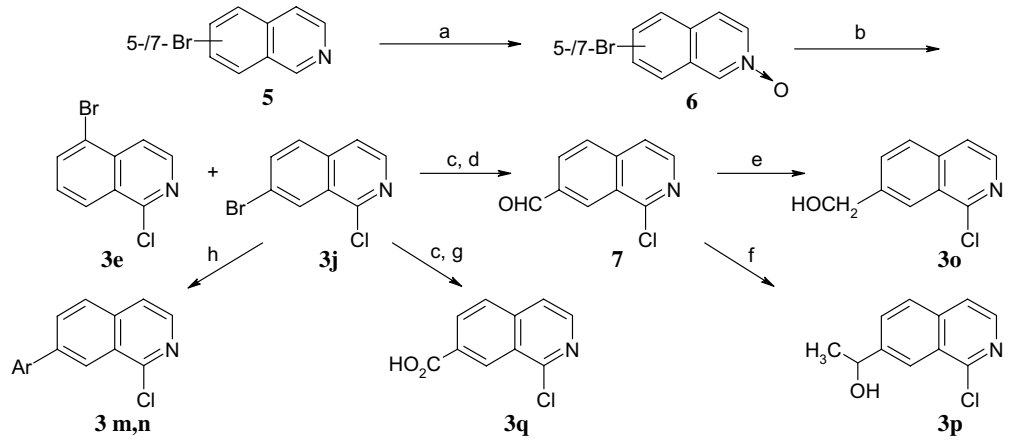
doi:10.1016/j.bmcl.2004.03.094



Scheme 2. Reagents and conditions: (a) 30% H_2O_2, AcOH, 80 °C; (b) $POCl_3$, CH_2Cl_2, reflux; (c) *n*-BuLi, THF/Et_2O (1:1), −78 °C; (d) DMF; (e) $NaBH_4$, MeOH; (f) MeLi, THF; (g) CO_2; (h) $Ar(BOH)_2$, tetrakis(triphenylphosphine)palladium(0), Na_2CO_3, H_2O, $MeO(CH_2)_2OMe$.

fractional crystallization from THF at −78 °C to give the individual isomers **3e** and **3j** (Scheme 2). The 7-bromo isomer **3j** was used for the preparation of further 7-substituted intermediates. Thus, treatment with *n*-BuLi and DMF gave the aldehyde **7**, which was converted to the alcohols **3o** and **3p**. The acid **3q** was prepared by successive treatment of **3j** with *n*-BuLi and CO_2. 7-Aryl substituted compounds **3m** and **3n** were prepared via Suzuki coupling of **3j** with the corresponding arylboronic acids.

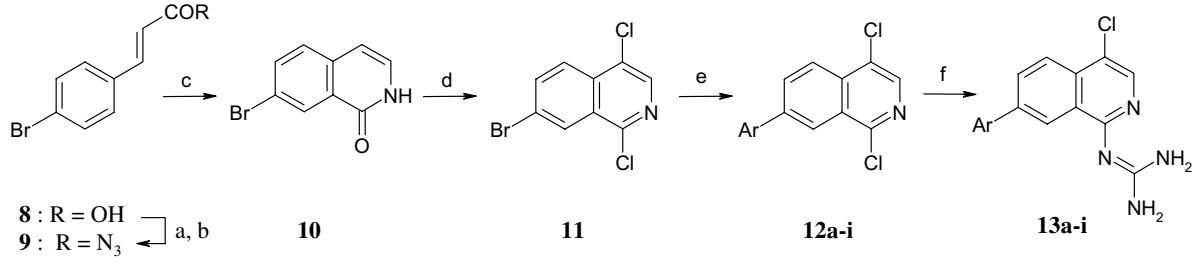
An alternative approach was used for the preparation of 7-substituted-4-chloro intermediates (Scheme 3). Conversion of 4-bromocinnamic acid to the acid chloride followed by treatment with sodium azide gave the azide **9**. This was submitted to a Curtius rearrangement (CAUTION, risk of explosion)[21] with concomitant cyclization to give the isoquinolin-1-one **10**, which was treated with PCl_5 to give the 1,4-dichloroisoquinoline intermediate **11**. This was used for preparation of a series of 7-aryl derivatives (**12a**–**i**) via Suzuki coupling with a variety of arylboronic acids. Treatment of the 1,4-dichloroisoquinolines **12a**–**i** with guanidine in DMSO gave the required isoquinolinylguanidine derivatives **13a**–**i**. Acid hydrolysis of the nitriles **13a**–**i** gave the corresponding acids **13j**–**l**.

An alternative approach was used to prepare 2-quinolinylguanidine **14** and 3-isoquinolinylguanidine **15**. In these cases, the corresponding amino compounds were treated with *N*,*N*′-bis(*t*-butoxycarbonyl)-*S*-methylisothiourea in the presence of mercury(II) chloride, followed by deprotection with trifluoroacetic acid.[10]

Compounds were tested for their ability to inhibit uPA, tPA and plasmin as described previously.[10] Results for simple isoquinolinylguanidine derivatives, expressed as a calculated K_i, are listed in Table 1, together with comparative figures for **1**.

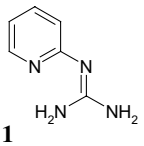
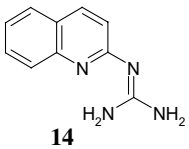
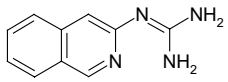
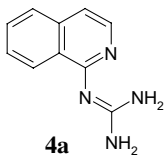
Benzo-fusion across the 3,4-positions of the pyridine ring to give the 1-isoquinolinylguanidine **4a** leads to a marked increase in potency compared with **1**, and fusion across the 4,5-positions as in 3-isoquinolinylguanidine **15** is also favourable.[30] A clear drop in potency is observed with 2-quinolinylguanidine **14**, which is consistent with our previous finding that a 6-substituent in the pyridine ring is unfavourable.[10] Compound **4a** also shows a reasonable level of selectivity with respect to tPA and plasmin. Based on these results, further SAR exploration was carried out in the 1-isoquinolinylguanidine series, and results for a range of analogues are summarized in Table 2.

From the results in Table 2 it is apparent that methyl substitution at the 4-position (**4b**) is tolerated but a chloro or bromo substituent is preferred (**4c** and **4d**). This parallels the situation in the pyridine series where 5-halo substitution also gave an increase in potency.[10] A small drop in potency results from introduction of



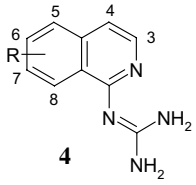
Scheme 3. Reagents and conditions: (a) $SOCl_2$, 23 °C; (b) NaN_3, Me_2CO, −10 to 0 °C; (c) Ph_2O, 270 °C (***Caution***, *risk of explosion*); (d) PCl_5, 140 °C; (e) $Ar(BOH)_2$, tetrakis(triphenylphosphine)palladium(0), Na_2CO_3, H_2O, $MeO(CH_2)_2OMe$; (f) Guanidine HCl, NaH, DMSO, 100 °C.

Table 1. Enzyme inhibition data for 2-pyridinylguanidine and benzo-fused analogues

	 **1**	 **14**	 **15**	 **4a**
uPA K_i (μM)	29	209	9.3	1.92
tPA K_i (μM)	a	367	a	126
Plasmin K_i (μM)	a	a	a	68

(a) <50% inhibition at 1 mM.

Table 2. Enzyme inhibition data for 1-isouinolinylguanidines



4

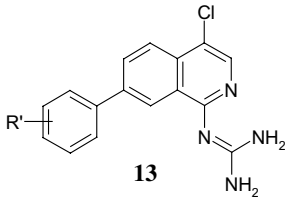
Compd	R	K_i (μM) or % inhibition		
		uPA	tPA	Plasmin
4a	H	1.92	126.2	68
4b	4-CH_3	1.40	a	a
4c	4-Cl	0.83	128.5	b
4d	4-Br	0.58	159.2	b
4e	5-Br	4.93	140.1	237.8
4f	5-OCH_3	3.62	b	c
4g	5-$OCH_2C_6H_5$	4.80	13	a
4h	5-C_6H_5	>100	123.9	47.5
4i	6-Br	2.34	b	131.4
4j	7-Br	0.80	95.1	50.8
4k	7-OCH_3	0.66	a	a
4l	7-$OCH_2C_6H_5$	4.80	13	a
4m	7-C_6H_5	0.40	123.9	47.5
4n	7-(3,4-OCH_2O)C_6H_4	0.34	d	12
4o	7-CH_2OH	1.30	a	d
4p	7-CH(OH)CH_3	0.64	a	24
4q	7-CO_2H	1.00	a	a

(a) <50% inhibition at 100 μM; (b) <50% inhibition at 1 mM; (c) <50% inhibition at 300 μM; (d) 55% inhibition at 100 μM.

5-substituents (**4e–g**), and 5-phenyl substitution is particularly detrimental (**4h**). The most favourable effects are seen with 7-substitution. Thus, the bromo and methoxy analogues **4j** and **4k** show an increase in potency, and aryl substitution as in **4m** and **4n** is particularly beneficial. Polar substitution, as in the alcohol **4o** and the carboxylic acid **4q** has no significant effect, but increasing the lipophilicity of the alcohol (**4p**) is beneficial. Some activity against tPA and plasmin was apparent with some compounds but, in most cases, selectivity for uPA was excellent.

From these results it was concluded that substitution at the 4-position of the isoquinoline ring with halogens and at the 7-position with lipophilic substituents, particularly aryl, is favourable. This further mirrored our previous work with the pyridylguanidines,[10] and suggested that further potency gains could be achieved by the substitution of the 7-phenyl substituent, which is summarized in Table 3.

Table 3. Enzyme inhibition data for 7-aryl-4-chloro-1-isoquinolinylguanidines



13

Compd	R′	K_i (μM) or % inhibition		
		uPA	tPA	Plasmin
13a	H	0.31	a	a
13b	4-CH_3	0.13	19	7.30
13c	2-OCH_3	0.10	15.5	14.5
13d	3-OCH_3	0.083	b	ND
13e	4-OCH_3	0.20	b	3.00
13f	3,4-(OCH_2O)	0.10	b	3.20
13g	3-CN	0.30	b	4.10
13h	4-CN	0.49	b	82% @ 10 μM
13i	2-OCH_3, 5-CN	0.18	b	a
13j	3-CO_2H	0.037	b	c
13k	4-CO_2H	0.082	b	65% @ 3 μM
13l	2-OCH_3, 5-CO_2H	0.009	b	4.75

(a) <50% inhibition at 30 μM; (b) <50% inhibition at 100 μM; (c) <50% inhibition at 10 μM.

A consistent additive effect upon the potency is seen with the 4-chloro substituent in the presence of a 7-aryl group (compare **13a** vs **4m**, and **13f** vs **4n**). The introduction of additional alkyl or alkoxy substitution in the phenyl ring is beneficial (**13b–f**). However, several of these compounds show significantly reduced selectivity with respect to plasmin. The most marked effect on uPA potency results from introduction of a carboxylic acid substituent as in **13j–l**. A *meta*-carboxylic acid gives the highest potency, and the 2-methoxybenzoic acid analogue **13k** is the most potent compound in the series. The structural similarity between **13j** and **13l** and the most potent compound in the pyridine series (**2**, K_i 0.17 μM), suggests that the pyridyls are functioning as ring-opened isoquinolines.[22]

In summary, we have found that 1-isoquinolinylguanidines are selective inhibitors of uPA, and that potency is enhanced by the presence of a 4-halo and a 7-aryl substituent. The carboxylic acid analogue **13j** (UK-356,202)

combines excellent potency and selectivity, and has been selected for clinical evaluation.

Acknowledgements

We thank Y. M. Ailwood, G. Bish, N. Smith and R. S. Strang for their assistance in preparing the compounds, M. F. Burslem, G. Easter and B. Williams-Jones for the biological data.

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22. Since completion of this work, X-ray data have been published[23–29], which can help to rationalize the activity of the compounds described here. Comparison of the X-ray crystal structures of the uPA inhibitors amiloride and phenylguanidine bound to re-engineered human uPA[23] showed that both bind in the S_1 pocket. Nienaber[24] proposed that while these two scaffolds do not overlap, their combination describes a naphthalene ring that could have good activity, and further, that a 4-Cl would enhance potency by occupying the $S_1\beta$ subsite. The SAR that we describe here system supports that prediction.
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