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3-Amidinophenylalanine-based Inhibitors of Urokinase

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Abstract: Synthesis and anti-uPA activity of a series of Nα-triisopropyl-phenylsulfonyl-protected 3-amidino-phenylalanine amides are described. We have explored SAR around the C-terminal amide part for inhibition of uPA, plasmin and trypsin. Modification of the amide part has been found to affect potency but not selectivity. With a K_i of 0.41 μM **2r-L** is one of the most potent uPA inhibitors described so far. The X-ray crystal structure of **2r-L** was solved in complex with trypsin, superimposed with uPA and the results suggest an unique binding mode of this inhibitor type.

Cellular invasiveness is a proteolytic process that contributes to a variety of physiological events, but also plays a decisive role in various disease processes. Although many details are not clear and the pathophysiological importance of the proteolytic activities have yet to be proven, there is strong evidence that the plasminogen activator urokinase (uPA) and the urokinase receptor exert a major role in these processes.[1,2] Therefore, potent uPA inhibitors may be useful as a pharmacological tool in the investigation of the enzyme's role in disease, especially in tumor invasion and metastasis. Furthermore, blocking cell surface proteolytic activities of uPA is an attractive goal in the development of antiinvasiveness drugs.[3]

Since urokinase is a trypsin-like serine protease, most uPA inhibitors described to date contain groups which mimic the basic side-chain of arginine. A first series was based on naphthamidines,[4] and a second group was derived from benzamidines and compounds containing two amidino moieties, where only a few derivatives showed K_i-values in the micromolar range.[4,5] A third series was described with substituted phenylguanidines;[6] in addition, moderate anti-uPA activity was found for the diuretic drug amiloride, a cyclic guanidino derivative.[7] Most recently bicyclic aromatic amidines containing a benzothiophene were reported: the compounds B428 and B623 inhibit uPA with a K_i of 0.53 and 0.16 μM, respectively, and represent the most potent competitive inhibitors of uPA described so far.[8] These analogous compounds were highly potent inhibitors of the activity of cell surface-bound uPA, cancer invasion and metastasis.[8-10]

Derivatives containing a benzamidine moiety as P1, e.g. 3- or 4-amidinophenylalanine, are useful key structures for the development of protease inhibitors of the trypsin family.[11-13] In particular, some piperidides and piperazides of 3-amidinophenylalanine N-terminally protected with a 2-naphthylsulfonyl (ßNAPS) residue

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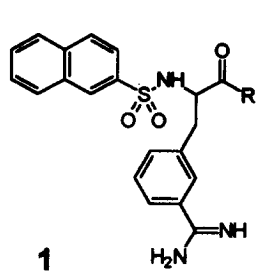
(formula 1) inhibit thrombin and trypsin with K_i-values in the nanomolar range, while the inhibition of uPA is relatively weak. The most potent uPA inhibitors were identified among compounds of formula 2 containing the bulky N-terminal triisopropyl-phenylsulfonyl (TIPPS) residue. SAR for inhibition of uPA, plasmin and trypsin will be presented.



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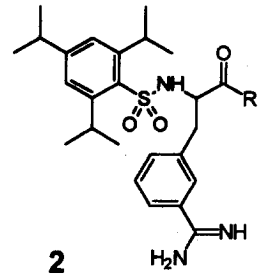

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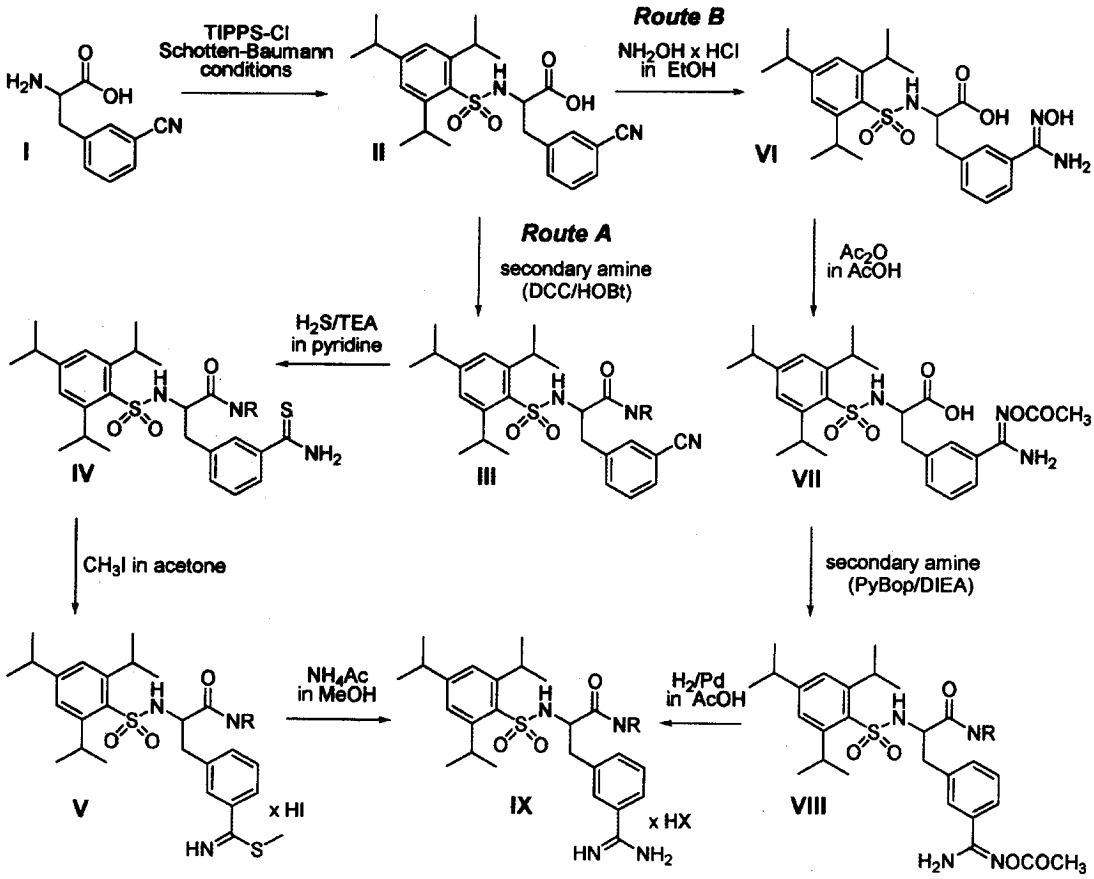
Chemistry

The inhibitors were synthesized as summarized in the scheme. Firstly, 3-cyanophenylalanine I (either in the racemic or in the L-form) was converted to the TIPPS derivative II. Two different strategies were used in



the following steps. In route A, **II** was first coupled with an appropriate secondary amine to give the intermediate **III**, which was converted into the thioamide **IV**, as previously described in detail.[12,13] S-Methylation resulted in the thioimidate hydroiodide **V**, which was converted to the final amidino-compound **IX**. Inhibitors containing a C-terminal free carboxylic acid were obtained by saponification of the respective methyl esters. In route B, **II** was converted to the corresponding amidoxime **VI**; treatment with acetic anhydride resulted in the O-acetylamidoxime **VII**.[14] **VII** was coupled with the appropriate amines to give **VIII**, and final hydrogenation resulted in **IX**. All end products were purified on Sephadex LH-20 or by preparative reversed phase HPLC.

Results and Discussion

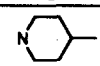
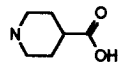
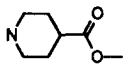
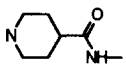
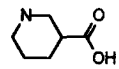
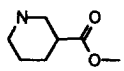
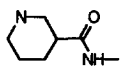
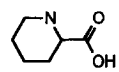
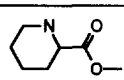
Several secondary amides of **2** were synthesized and compared with the related ßNAPS-protected derivatives of **1** (Table 1). While Nα-TIPPS-substitution has only a slight effect on the inhibition of plasmin and trypsin, most of the TIPPS-protected derivatives inhibit uPA with higher affinity compared with the ßNAPS-substituted compounds (e.g. 11-fold in case of **1e** and **2e**), the most potent inhibitor was the homopiperidide **2c** (K_i of 4.4 μM).

Table 1. Inhibition of uPA, Plasmin and Trypsin by Secondary Amides of ßNAPS- and TIPPS-Protected 3-Amidinophenylalanine, the Compounds are Racemates.

Compound	R	K_i (μM)		
		uPA	Plasmin	Trypsin
1a		73	12	0.44
2a		19	9.6	3.9
1b		38	7.0	0.33
2b		5.1	2.6	1.3
1c		28	6.6	0.74
2c		4.4	2.8	0.95
1d		19	7.8	0.61
2d		7.8	3.2	0.83
1e		58	7.7	0.21
2e		5.4	4.5	0.49
1f		48	10.2	0.22
2f		12	7.1	0.98

Further analogs of the piperidide **2b** (Table 2) and the piperazide **2f** (Table 3) were synthesized to enhance the anti-uPA activity. Incorporation of 4-methylpiperidide (**2g**) or methyl esters of nipecotic and isonipecotic acids (compounds **2i** and **2l**) slightly increased the anti-uPA activity; in contrast, introduction of a C-terminal D-pipecolic acid methyl ester (**2o**) remarkably reduced the activity. Differentiation was also found with the free carboxylic acid derivatives. Here, the 2-substituted D-pipecolic acid derivative **2n** was the most potent compound with a micromolar K_i, while the iso-nipecotic and the nipecotic acid derivatives **2h** and **2k** were less active. Several other ester and amide derivatives of nipecotic and iso-nipecotic acid were synthesized, but none of the analogs showed better inhibitory activity as shown by the methylamides **2j** and **2m** (Table 2).

Table 2. Inhibition of uPA, Plasmin and Trypsin by Substituted Piperidides of Structure **2**, Compounds **2g-2m** are Racemates, **2n** and **2o** are the L-3-Amidinophenylalanine D-Pipecolic Acid Derivatives.

Compound	R	K_i (μM)		
		uPA	Plasmin	Trypsin
2g		2.0	4.0	0.69
2h		52	12	6.4
2i		1.3	1.4	0.27
2j		4.8	2.5	0.36
2k		17	8.2	2.4
2l		2.5	1.0	0.36
2m		12	0.40	0.79
2n		1.3	1.5	0.5
2o		33	1.0	1.4

In Table 3 the K_i-values of several substituted piperazides are summarized. Like the methyl and the methylsulfonyl derivatives **2p** and **2t**, other alkylated or sulfonylated piperazides are poor inhibitors of uPA. However, acetylation of the piperazine ring (**2s**) or substitution by an alkyloxycarbonyl residue (**2q**, **2r**) increased the anti-uPA activity compared to the free piperazide **2f**. Comparing the K_i-values determined for the other enzymes tested, the selectivity of inhibition is relatively low for the piperazides, as seen also for the piperidides.

X-ray crystal structures of trypsin and thrombin[15,16] and synthesis of the pure enantiomers[12,13] showed that optimal insertion of the benzamidine moiety into the specificity pocket requires an L-configuration of the central 3-amidinophenylalanine residue in ßNAPS derivatives. This could also be confirmed for the inhibition of uPA by Nα-TIPPS-protected inhibitors. The K_i for inhibition of uPA by the L-enantiomer of the most potent compound of our series, the ethoxycarbonyl derivative **2r**, was nearly half that of the racemate. With a K_i of 0.41 μM for inhibition of uPA, **2r-L** is only 3 times less potent than the most active benzothiophene derivative designed by TOWLE et al.[8] However, in contrast to the benzothiophene-derived inhibitor, **2r-L** exhibits low specificity as it inhibits also several other trypsin-like enzymes with comparable potency (Table 4).

Table 3. Inhibition of uPA, Plasmin and Trypsin by Piperazides of Structure **2**, the Compounds are Racemates.

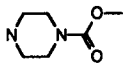
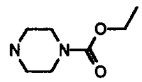
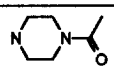
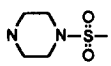
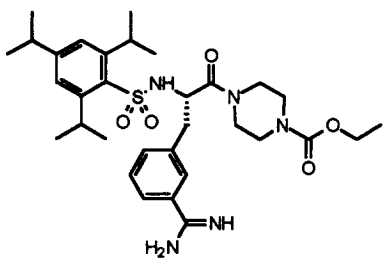
Compound	R	K_i (μM)		
		uPA	Plasmin	Trypsin
2p		52	21	3.0
2q		0.86	1.8	0.11
2r		0.96	1.2	0.12
2s		3.5	3.0	0.22
2t		19	8.0	0.62

Table 4. Inhibition of Several Trypsin-like Ezymes by the L-Enantiomer of Compound **2r**.



Enzyme	K_i (μM)
uPA	0.41
sc-tPA	4.9
Plasmin	0.39
Thrombin	0.49
Factor Xa	1.7
Protein Ca	2.3
Plasma kallikrein	7.2
Trypsin	0.037
Tryptase	6.3

The X-ray crystal structure of uPA irreversibly inhibited by Glu-Gly-Arg chloromethyl ketone was published recently.[17] As we have been unable to crystallize the inhibitors described in the paper with uPA, we have studied the trypsin complex formed with several TIPPS-derived analogs.[18] Using the uPA structure, reliable models of the binding of Nα-TIPPS-protected inhibitors within uPA could be established. Figure 1 shows the structure of **2r-L** within the active site of uPA. Unlike other serine proteinase-inhibitor complexes, the inhibitor with the bulky TIPPS residue can not occupy the „aryl-binding site" which is partially filled in uPA by an insertion of two amino acids (Thr97A and Leu97B). Instead, the inhibitor protrudes from the specificity pocket with an isopropyl group preventing the TIPPS residue from entering the aryl binding site. This arrangement avoids a potential clash of the inhibitor with the extended „97-insertion" of uPA.

The proposed unique binding mode of the TIPPS derivatives, which enables binding without any steric hindrance, is presumably a key characteristic of this type of uPA inhibitors. The modeled structures are used to design more potent and selective uPA inhibitors, and the effects of the uPA inhibitor **2r-L** on cancer invasion and metastasis are subject of further investigations.

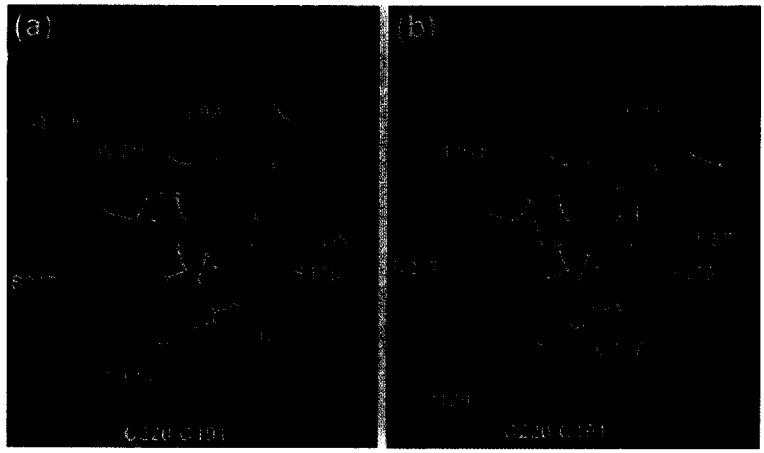


Figure 1. (a) Experimental ($2F_o$-F_c) electron density of **2r-L** (white) in bovine trypsin (yellow).[18] The inhibitor resembles a bush that has been planted in the specificity pocket. **(b)** Transfer of **2r-L** to the active site of uPA (orange) based upon the X-ray structure of uPA inhibited by Glu-Gly-Arg chloromethyl ketone.[17] The side chain of Gln192 has been rotated to its position in the trypsin complex, and the ethyloxycarbonyl group has been rotated by 180° to avoid a clash with His99. The molecular configuration obviates steric hindrance between the extended 97 loop of uPA (top) and the TIPPS and piperazide portions of the inhibitor.

Methods

The measurements for determination of inhibition constants were carried out on a microplate reader (MR 5000, Dynatech, Denkendorf, Germany) at 25 °C as described previously.[13]

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References

1. Andreasen, P. A.; Kjøller, L.; Christensen, L.; Duffy, M. J. *Int. J. Cancer* **1997**, *72*, 1.
2. Edwards, D. R.; Murphy, G. *Nature* **1998**, *394*, 527.
3. Jankun, J.;Keck, R. W.; Skrzypczak-Jankun, E.; Swiercz, R. *Cancer Res.* **1997**, *57*, 559.
4. Stürzebecher, J.; Markwardt, F. *Pharmazie* **1978**, *33*, 599.
5. Geratz, J. D.; Shaver, S. R.; Tidwell, R. R. *Thromb. Res.* **1981**, *24*, 73.
6. Yang, H.; Henkin, J.; Kim, K. H.; Greer, J. *J. Med. Chem.* **1990**, 33, 2956.
7. Vassalli, J. D.; Belin, D. *FEBS Letters* **1997**, *214*, 187.
8. Towle, M.. J.; Lee, A.; Maduakor, E. C.; Schwartz, E.; Bridges, A. J.; Littlefield, B. A. *Cancer Res.* **1993**, *53*, 2553.
9. Rabbani, S. A.; Harakidas, P.; Davidson, D. J.; Henkin, J.; Mazar, P. *Int. J. Cancer* **1995**, *63*, 840.
10. Alonso, D. F.; Tejera, A. M.; Farias, E. F.; Joffé, E. B. D.; Gomez, D. E. *Anticancer Res.* **1998**, *18*, 4499.
11. Markwardt, F.; Wagner, G.; Stürzebecher, J.; Walsmann, P. *Thromb. Res.* **1980**, *17*, 425.
12. Stürzebecher, J.; Prasa, D.; Wikström, P.; Vieweg, H. *J. Enzyme Inhibition* **1995**, *9*, 87.
13. Stürzebecher, J.; Prasa, D.; Hauptmann, J.; Vieweg, H.; Wikström, P. *J. Med. Chem.* **1997**, *40*, 3091.
14. Judkins, B. D.; Allen, D. G.; Cook, T. A.; Evans, B.; Sardharwala, T. S. *Synthetic Communications* **1996**, *26*, 4351
15. Turk, D.; Stürzebecher, J.; Bode, W. *FEBS Letters* **1991**, *287*, 133.
16. Brandstetter, H.; Turk, D.; Hoeffken, H. W.; Grosse, D.; Stürzebecher, J.; Martin, P. D.; Edwards, B. F. P.; Bode, W. *J. Mol. Biol.* **1992**, *266*, 1085.
17. Spraggon, G.; Phillips, C.; Nowak, U. K.; Ponting, C. P.; Saunders, D.; Dobson, C. M.; Stuart, D. I.; Jones, E. *Structure* **1995**, *3*, 681.
18. Renatus, M.; Bode, W.; Huber, R.; Stürzebecher, J.; Stubbs, M. T. *J. Med. Chem.* **1998**, 41, 5445.