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Structure-Based Design, Synthesis and SAR of a Novel Series of Thiopheneamidine Urokinase Plasminogen Activator Inhibitors

Nalin L. Subasinghe,* Carl Illig, James Hoffman, M. Jonathan Rudolph, Kenneth J. Wilson, Richard Soll, Troy Randle, David Green, Frank Lewandowski, Marie Zhang, Roger Bone, John Spurlino, Renee DesJarlais, Ingrid Deckman, Christopher J. Molloy, Carl Manthey, Zhau Zhou, Celia Sharp, Diane Maguire, Carl Crysler and Bruce Grasberger

3-Dimensional Pharmaceuticals Inc., 665 Stockton Drive, Exton, PA 19341, USA

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Abstract—The serine protease urokinase plasminogen activator (uPA) is thought to play a central role in tumor metastasis and angiogenesis. Molecular modeling studies suggest that 5-thiomethylthiopheneamidine inhibits uPA by binding at the S1 pocket of the active site. Further structure based elaboration of this residue resulted in a novel class of potent and selective inhibitors of uPA.

The serine protease urokinase plasminogen activator (uPA) is thought to play a central role in tumor metastasis and angiogenesis.[1] Proteolytically active uPA is a disulfide-linked two-chain protein, which is generated from a largely proteolytically inactive 54-kDa pro-uPA by hydrolysis of the Lys158-Ile159 peptide bond.[2] The primary function of uPA is to convert plasminogen to plasmin,[3] which can digest components of the extracellular matrix and basement membrane either directly or indirectly by activating pro-MMPs. Extracellular matrix remodeling via proteolysis is a key step in tumor metastasis and angiogenesis. An up-regulation of uPA in tumor versus normal tissue has been observed for a wide variety of human cancers.[4,5] Furthermore, many studies have shown that the inhibition of urokinase enzyme activity can reduce tumor growth and/or metastasis.[5] Given these observations, inhibition of plasminogen activation by uPA appears to be an attractive approach for the therapeutic intervention of tumor growth and metastasis.

There are reports of nonpeptidic, reversible inhibitors of uPA from as far back as the late 1950's. These small molecule inhibitors include benzamidines, phenylguanidines, acylguanidines and bisbenzamidines.[6] The best of these early uPA inhibitors have μM potencies and poor selectivity. Several novel uPA inhibitors with nM potency and selectivity towards uPA have been described in the recent literature. These include benzothiopheneamidines[7] (Eisai), cyclohehylthiopheneamidines[8] (Fujisawa), naphthylamidines[9] (Abbott), and isoquinolynylguanidines[10] (Pfizer). Here, we describe the design and synthesis of a novel series of potent and selective uPA inhibitors.

Our efforts began with the search for a novel basic residue that would potentially bind to the S1 site[11] of uPA. After screening several hundred amidines, guanidines, and amines, 5-methylthiothiopheneamidine **1** ($K_i = 6$ μM) was selected as a suitable P1 residue for structure-based design. Molecular modeling of **1** and the benzothiophene B623 (Eisai, $K_i = 0.53$ μM) within the enzyme active site suggested a common binding mode for both compounds. The arylamidine portion of both compounds appears to bind within the S1 pocket where the amidine moiety forms a salt bridge with Asp189 of uPA. Furthermore, these studies suggested that the styryl side chain of B623 would fit into a small hydrophobic pocket formed by Gly218, Ser146, and Cys191–Cys220 disulfide bridge.[12] Substitutions at the 4-position of **1** occupy the same area of space as the styryl side chain of B623 and could potentially bind within this

*Corresponding author. Tel.: +1-610-458-6066; fax: +1-610-458-8249; e-mail: nalin@3dp.com

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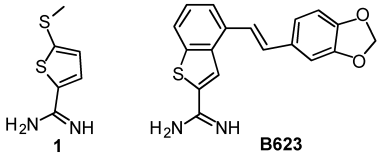
proximal aryl-binding pocket. To explore this possibility a series of 4-substituted thiopheneamidines was synthesized (Table 1). The more promising 4-substituted thiopheneamidines were further elaborated to identify structure–activity relationships.

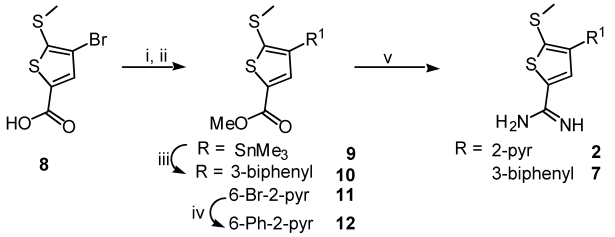


4-(2-Phenyl-6-pyridyl)thiopheneamidine **2** and 4-(*m*-biphenyl)-thiopheneamidine **7** were synthesized as summarized in Scheme 1. The dilithium salt, generated by treating methyl 4-bromo-2-methylthiothophene-2-carboxylate **8** with 2 equiv of *n*BuLi, was converted to the stannane **9** by treating with trimethyltin chloride.

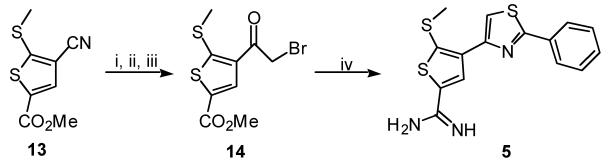
The stannane was treated with 3-phenylbromobenzene and 2,6-dibromopyridine under Stille[13] coupling conditions to give the biphenylthiophene **10** and (6-bromopyridyl)thiophene **11**, respectively. The (6-bromopyridyl)thiophene **11** was treated with phenylboronic acid under Suzuki[14] coupling conditions to give compound **12**. The esters **11** and **12** were converted to the corresponding amidines **2** and **7** by treating with trimethylaluminum and ammonium chloride in refluxing toluene.[15]

4-Phenylthiopheneamidine **3** and 4-(4-methylthiazolyl)thiopheneamidine **4** were prepared by subjecting the corresponding esters (Maybridge Chemical Company, Cornwall, UK) to the amidination conditions described in Scheme 1. 4-(2-Phenythiazolyl)-thiopheneamidine **5** was synthesized by treating the bromoketone **14** with phenylthioamide (Scheme 2).[16]

The nitrile **13** was selectively hydrolyzed to the acid by heating with tetrafluorophthalic acid in a sealed tube for 72 h.[17] The acid was converted to the acid chloride and then treated with trimethylsilyldiazomethane to form the diazoketone.[18] The diazoketone was treated with HBr in AcOH to give the bromoketone **14**. 4-Alkyl and 4-arylthiazolylthiopheneamidines listed in Tables 2 and 3 were synthesized according to Scheme 3. The nitrile **13** was treated with H_2S[19] to form the thioamide **15**, which was reacted with bromoketones to give a variety of thiazolylthiophene esters. These thiazolylthiophenes were converted to the corresponding amidines in a

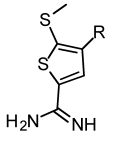


Scheme 1. Reagents and conditions: (i) BuLi (2 equiv), $ClSnMe_3$; (ii) $TMSCHN_2$; (iii) RBr, Pd; (iv) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, aq DMF, 80 °C; (v) $AlMe_3/NH_4Cl$, toluene, reflux.



Scheme 2. Reagents: (i) Tetrafluorophthalic acid, Δ; (ii) $(CO)_2Cl_2$, DMF cat, CH_2Cl_2, 3 h; (iii) (a) $TMSCHN_2$, CH_3CN 4 h; (b) 30% HBr/AcOH 1 h; (iv) $PhCSNH_2$, reflux, acetone.

Table 1. uPA inhibitory activity of 4-substituted thiopheneamidines



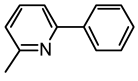
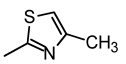
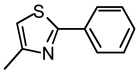
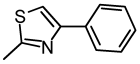
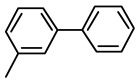
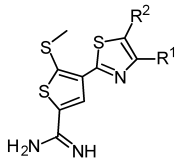
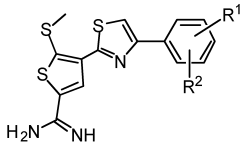
Compound	R	K_i (μM)
2		>28
3		0.6
4		1.0
5		0.2
6		0.09
7		>34

Table 2. SAR of thiazole substitution

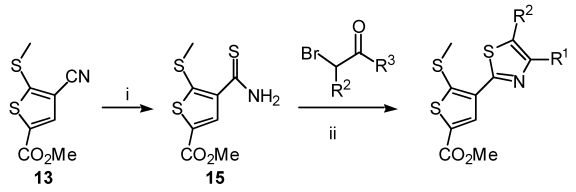


Compound	R^1	R^2	K_i (nM)
16	Thiophene-2-yl	H	89
17	Benzothiophen-3-yl	H	141
18	2-Naphthyl	H	138
19	5,6,7,8-Tetrahydronaphth-2-yl	H	145
20	3,4-(OCH_2O)Ph	H	91
21	3,4-(OCH_2CH_2O)Ph	H	108
22	3,4-($OCH_2CH_2CH_2O$)Ph	H	100
23	2-Cl-3-Pyridyl	H	154
24	4-Cl-3-Pyridyl	H	164
25	2-Cl-4-Pyridyl	H	120
26	CH_3	H	815
27	$PhOCH_2$	H	178
28	Cyclohexyl	H	641
29	$PhCH_2$	H	470
30	Ph	CH_3	305
31	Ph	Ph	982

Table 3. SAR of phenyl substitution for compound **6**



Compound	R^1	R^2	K_i (nM)
32	4-CH_3	H	94
33	4-CH_3PhNHO_2S	H	272
34	4-Cl	H	99
35	4-Phenyl	H	1740
36	4-NO_2	H	270
37	4-CH_3O	H	327
38	4-CH_3SO_2NH	H	94
39	2-CH_3O	H	130
40	2-CH_3	H	277
41	3-CH_3O	H	115
42	3-OH	H	86
43	3-Br	H	442
44	3-CH_3	H	546
45	3-CH_3OCOCH_2O	H	44
46	3-(4-F-PhCONH)	H	47
47	2-CH_3O	4-CH_3O	152
48	2-Cl	4-Cl	287
49	3-CH_3	4-OH	44
50	3-NO_2	4-Cl	161



Scheme 3. Reagents: (i) H_2S, Et_3N, anhyd MeOH, 18 h; (ii) acetone, reflux, 5 h.

manner similar to that described in Scheme 1. Bromoketones, which were not commercially available, were generated either by brominating the corresponding ketones[18] or by treating the corresponding carboxylic acid in a manner similar to that shown in Scheme 2 (steps ii and iii).

The inhibition constants for several 4-substituted thiopheneamidines are shown in Table 1.[20] Phenyl (**3**) and thiazolyl (**4**) substitution at the 4-position of the thiophene ring increases activity by 10- and 8-fold, respectively. Biphenyl (**7**) substitution resulted in an inactive compound. Replacing the methyl group at the 4-position of the thiazole in compound **4** with a phenyl group (**6**) resulted in a further 8-fold increase in activity. Based on these results, 4-(2-thiazolyl)thiopheneamidine was chosen as a scaffold for further elaboration. Results in Table 2 show the effect of substitutions on the thiazole ring. 4-Thiophene (**16**) and 4-(3,4-dioxalanyl)phenyl (**20**) substitution gave potency that was equivalent to the phenyl analogue (**6**). Other aryl substitutions did not provide a significant enhancement in activity. In contrast, 4-alkyl substitutions gave less active compounds.

Alkyl and aryl groups at the 5-position of the thiazole ring also gave less active compounds. The effect of further

Table 4. Selectivity for representative compounds

Compound	K_i (μM)				
	Thrombin	Plasmin	Chymotrypsin	tPA	Trypsin
20	$>29^a$	10.8	—	1.7	0.88
41	$>30^a$	6.9	$>30^a$	1.9	0.86
42	$>25^a$	32.1	$>31^a$	1.7	0.93

[a]No observable inhibition at this screening concentration.

substitution on the phenyl ring of compound **6** was also evaluated (Table 3). Small hydrophobic residues such as Me (**32**) and Cl (**34**) in the *para* position can be accommodated with no loss in potency.

There is no significant difference between electron-withdrawing and electron-donating residues. Even though bulky arylsulfonamides at the *para* position (**38**) retain activity, the rigid phenyl substitution (**35**) results in a 10-fold loss of activity. While hydrophobic groups at the *meta* position attenuated activity, *m*-aryl-carbonylamino (**46**) and *m*-(methoxycarbonyl)-methoxy (**45**) substitutions gave a 2-fold increase in activity.

Selected compounds were also tested for their inhibitory activity against other serine proteases. Activities for three representative compounds are shown in Table 4. Compound **42** has approximately 300-fold or greater selectivity over thrombin, plasmin, and chymotrypsin, and also has 20- and 10-fold selectivity over tPA and trypsin, respectively.

In conclusion, starting from a weakly active P1 scaffold, we have constructed a novel series of potent and selective inhibitors of human uPA. These compounds have nanomolar potencies and show inhibitory activity in cell-based assays[21] of tumor metastasis. Further results from structure-based optimization and in vivo biological results will be reported in due course.

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11. The binding site for a polypeptide substrate consists of a series of subsites within the enzyme, which are occupied by the amino acid residues. By convention these sites are labeled S_1, S_2, etc., counting from the carboxy residue of the scissile amide bond towards the N-terminus of the substrate and S_1', S_2', etc., counting from the amino residue of the scissile bond towards the C-terminus of the substrate. The corresponding amino acid residues of the substrate are labeled P and P′ in the same way.
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