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Synthesis of Thiophene-2-carboxamidines Containing 2-Aminothiazoles and their Biological Evaluation as Urokinase Inhibitors

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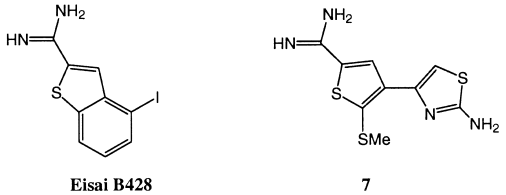
Abstract—The serine protease urokinase (uPa) has been implicated in the progression of both breast and prostate cancer. Utilizing structure based design, the synthesis of a series of substituted 4-[2-amino-1,3-thiazolyl]-thiophene-2-carboxamidines is described. Further optimization of this series by substitution of the terminal amine yielded urokinase inhibitors with excellent activities.

The progression of cancer cell invasion and metastasis is hinged on the ability of tumor cells to produce and recruit proteolytic enzymes. Although not well understood, there is a growing body of evidence that the plasminogen activation enzyme urokinase (uPA) and its receptor (uPAR) exert a major role in the pathogenesis of cancer.[1–4] Thus, the correlation of metastasis with increased levels of uPA and uPAR has initiated research into inhibition of the plasminogen activation system as a novel therapeutic target for cancer.[5]

Recently, two publications have appeared that report the X-ray crystallographic structure of variants of uPA bound to small molecule inhibitors.[6,7] Because urokinase is a trypsin-like serine protease, the majority of uPA inhibitors reported to date are based upon either amidine or guanidine functionality, which form a salt bridge with the Asp189 in the S1-binding pocket of uPA.[8–10] A series of uPA inhibitors based upon a benzothiophene scaffold was prepared by researchers at Eisai.[9] Those substituted at the 4-position, such as the Eisai B428 iodo-derivative gained notable in vitro ($K_i = 0.32\,\mu M$) activity (Fig. 1). Screening of amidine libraries showed 2-amidino-5-thiomethyl thiophene to be an active ($K_i = 6\,\mu M$) uPA inhibitor, whose potency could be increased by aryl or heterocycle substitution at the 4-position of the thiophene ring. For example, compound **7** was more potent with a $K_i = 3.90\,\mu M$. Molecular modeling of the Eisai compounds and compound **7** further supported the hypothesis that substitution at the 4-position of the thiophene would be the most advantageous for further lead optimization. We envisioned that substitution from the 2-aminothiazole of the synthetically accessible thiophene **7** would enable us to access the same proximal binding pocket as the 4-substituted Eisai series.[11] In fact, a recent disclosure of an Eisai B428-uPA crystal structure clearly shows the iodo-substitution vector of Eisai B428 is directed towards the proximal pocket (referred to as $S_1\beta$, see ref 6). The elaboration of the lead compound **7** is described within.



Eisai B428 **7**

Figure 1. The structures of the Eisai benzothiophene amidine and scaffold 4-(2-amino(1,3-thiazol-4-yl))-5-methylthiothiophene-2-carboxamidine **7**.

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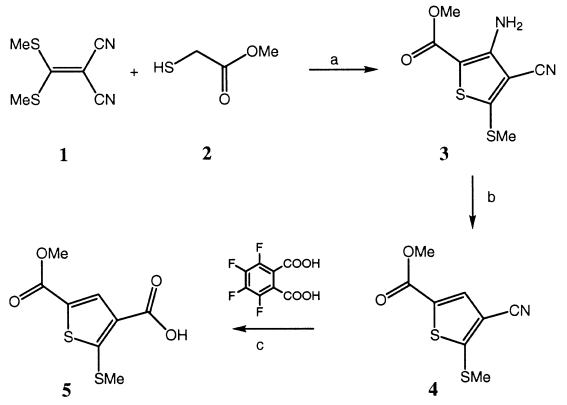
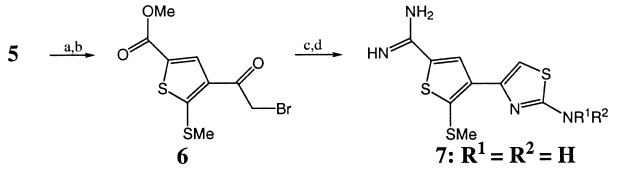
The highly substituted thiophene nucleus was prepared in one step by the condensation of dinitrile **1** with methylthioglycolate **2** to afford the multi-functionalized aminothiophene **3** (Scheme 1).[12] Deamination of the free amino group with isoamyl nitrite in DMF solution at 70 °C cleanly gave the properly substituted precursor **4**. After some experimentation, a hydrolysis of the nitrile to the carboxylate **5**, which left the ester functionality intact, was achieved using the procedure of Gribble and co-workers.[13] The acid **5** could be converted to the α-bromoketone **6** by a modified diazotization–bromination procedure developed in our laboratories.[14] The requisite aminothiazoles were then synthesized by reacting compound **6** with the appropriate 1-substituted or 1,1-disubstituted thiourea (prepared via their isothiocyanates) using standard Hantzch conditions.[15–17] It is noteworthy that the thiazole products are isolated as their hydrobromide salts by simple filtration and no chromatographic purification is required for any step in this synthesis. Finally, the conversion of the esters to the amidines **7**–**47** were accomplished with the $NH_4Cl/AlMe_3$ reagent[18] in parallel fashion employing a Fisher Scientific dry bath incubator equipped with an aluminum heating block (Scheme 2).

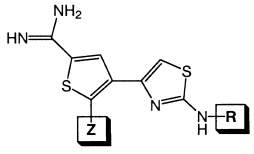


Scheme 1. Synthesis of thiophene nucleus. (a) triethylamine, MeOH; (b) isoamyl nitrite, DMF, 70 °C; (c) neat, 160 °C, 72 h.



Scheme 2. (a) Oxalyl chloride, CH_2Cl_2; (b) $TMSCHN_2$, CH_3CN, 30% HBr–acetic acid; (c) thiourea, acetone, 70 °C; (d) NH_4Cl, $AlMe_3$, toluene, 118 °C.

Table 1. Measured uPA inhibition of alkyl-aryl substituted thiophene-2-aminothiazoles



Compd	R Group	Z Group	K_i (μm)
7	H	SCH_3	3.90
8	H	CH_3	1.62
9	$-(CH_2)_2$-morpholine	SCH_3	1.40
10	$-(CH_2)_2$-piperidine	SCH_3	1.60
11	$-CH_2$-4-tolyl	SCH_3	0.64
12	$-(CH_2)_3$-phenyl	SCH_3	0.35
13	Phenyl	CH_3	0.17
14	3,4,5-Trimethoxyphenyl	CH_3	1.11
15	$-CH(C_6H_5)_2$	SCH_3	1.18

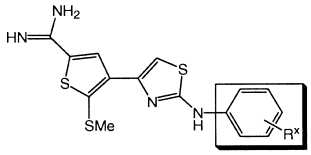
The replacement of the thiomethyl by methyl in the thiophene derivatives **8**, **13**, **14**, and **44** was also performed for SAR comparison using chemistry recently communicated from our laboratories.[19]

The effect of an aliphatic spacer between the amino thiazole and either an aliphatic or an aromatic termini was examined (see Table 1).[20] While the morpholine and piperidine derivatives **9** and **10** enhance the activity slightly, aliphatic tethers between the aminothiazole and aryl groups result in significant improvement (see **11** and **12**). The unsubstituted methyl derivative **8** shows a moderate increase in potency over its *S*-methyl counterpart **7**. The less bulky phenyl derivative **13** is a more active inhibitor than the trimethoxyphenyl compound **14**. An improvement in activity is also achieved relative to compound **7** by increasing the steric bulk of the proximal substituent, such as in diphenylmethyl derivative **15**.

Encouraged by these results, a library of alkyl, halogen, and heteroatom substituted phenyl derivatives **16**–**33** was synthesized and assayed against urokinase (Table 2).

It is immediately apparent that single aryl substitution on the aminothiazole results in a series of compounds with nanomolar activity. Only compounds **20**, **27**, **29**, and **31** possessed measured K_i values of greater than 1 μM, but all still more potent than **7**. A wide range of substitution and functionality is tolerated, as well as sterically demanding substrates such as **25**, **31**, and **32**, although the trimethoxyphenyl substituent is more active in the thiomethyl series (compare compound **32** to the methyl derivative **14**).

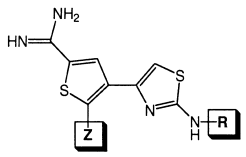
Table 2. Measured uPA inhibition of substituted aryl aminothiazole thiophene



Compd	Aryl group	K_i[a]	Compd	Aryl group	K_i
16	Phenyl	0.20	**25**	2-Isopropyl	0.70
17	3-Methoxy	0.28	**26**	2-Bromo	0.28
18	4-Methoxy	0.28	**27**	3-Oxyacetamide	1.02
19	4-Benzyloxy	0.18	**28**	2,5-Dimethoxy	0.66
20	2-Chloro	1.46	**29**	2,6-Dichloro	1.5[b]
21	2-Fluoro	0.24	**30**	2-Bromo-4-methyl	0.43
22	2-Methyl-4-chloro	0.28	**31**	2,4,5-Trimethyl	1.10
23	2,3-Dimethyl	0.28	**32**	3,4,5-Trimethoxy	0.16
24	2-Methyl-3-chloro	0.44	**33**	4-Dimethylamino	0.31

[a]Activity is expressed in micromolar units.
[b]Compound **29** possessed only limited solubility in DMSO.

Table 3. Measured uPA inhibition of bicyclic substituted thiophene-2-aminothiazoles



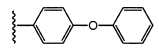
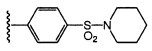
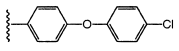
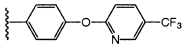
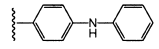
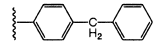
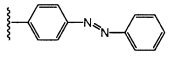
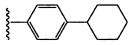
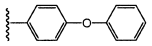
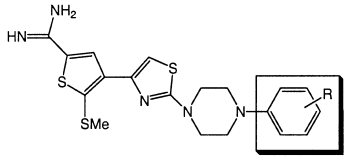
Compd	R Group: Z = SMe	K_i (μM)	Compd	R Group: Z = SMe	K_i (μM)
34		0.06	**40**		0.40
35		0.11	**41**		0.91
36		0.07	**42**		0.52
37		0.18	**43**		0.50
38		0.18		R Group: Z = Me	
39		0.11	**44**		0.08

Table 4. Measured uPA inhibition of piperizinyl substituted thiophene-2-aminothiazoles



Compd	R Group	K_i (μm)
45	3-Methoxy	1.84
46	4-Methoxy	0.48
47	2-Fluoro	3.06

It seemed evident from these results that considerable steric flexibility and therefore a variety of scaffolds could be tolerated in the proximal binding region. We next examined the effect of biaryl ether substituents and other bicyclic proximal pocket pharmacophores (Table 3). An improvement in activity is seen for this class of compounds. Except for compound **41**, all of the compounds in this group displayed K_i values of 0.52 μM or less with the most active compound being biaryl ether **34** ($K_i = 0.06$ μm). The methyl derivative **44** did not show a significant difference from **34** in activity. The boost in potency of these compounds in relation to the simple phenyl derivative **13** may be attributable to a more extended, rigid conformation that allows better van der Waals surface contacts with the proximal binding pocket of urokinase.

Several piperazine derivatives **45**–**47** were also prepared and tested (Table 4). Of the three compounds, only the *ortho*-fluoro derivative **47** does not show significant improvement over the lead compound **7**. This last set of compounds demonstrates the versatility of the aminothiazole as a proximal binding pocket scaffold in that primary, secondary, and tertiary 2-aminothiazoles all show uPA inhibitory activity.

In conclusion, it has been demonstrated that the lead compound **7** is an effective template for probing SARs of the proximal binding pocket of urokinase. A significant gain in potency can be realized by both aryl and alkyl substitution from the 2-aminothiazole group. These compounds can be prepared utilizing parallel synthetic methods starting from compound **6**. Also, replacement of the thiophene *S*-methyl with a methyl group is well tolerated. Presumably, the methyl and *S*-methyl derivatives bind very similarly within the S1-binding region. The use of larger, more rigid hydrophobic pharmacophores gave the best activities. The increase in potency observed in our 2-aminothiazole thiophene amidine series that result from substitution at the thiophene 4-position allows a compelling argument to be made that the proximal binding pocket can be exploited for further inhibitor optimization.

References and Notes

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16. A representative synthesis of a non-commercially available thiourea (4-(4-chlorophenoxy)-phenylthiourea), precursor for compound **35** is as follows: To a 20 mL biphasic mixture of $CHCl_3$/satd $NaHCO_3$ (1:1, v/v) was added 4-phenoxy-4-chloroaniline hydrochloride (520 mg, 2.03 mmol). Thiophosgene (185 μL, 2.43 mmol) was then added dropwise as a 5 mL solution in $CHCl_3$ via an addition funnel. The mixture was stirred vigorously for 30 min, at which time the layers were separated. The $CHCl_3$ layer was washed with brine (2×10 mL), dried (Na_2SO_4) and concentrated in vacuo to give 416 mg (78%) of 4-phenoxy-4-chlorophenylisothiocyanate as a nearly colorless oil. The oil was dissolved in 3 mL of MeOH and then treated with 3 mL of a 2 M solution of NH_3 in MeOH. The flask was sealed and allowed to stir for 12 h, at which time the flask was cooled in an ice-bath, and the off-white precipitate was filtered, washed with ether, and dried to afford 326 mg (79%, 58% over 2 steps) of the title compound as a white, crystalline solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.99–7.03 (m, 4H), 7.37–7.43 (m, 4H), 9.65 (s, 1H); MS (ESI) m/z calcd for $C_{13}H_{11}ClN_2OS$, 279.03 (M + H), found 279.4.
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20. Human kidney cell urokinase was purchased from Sigma Chemical Co. (St. Louis, MO). In a typical K_i determination, into each well of a 96-well plate is pipetted 280 μL of *N*-CBz-Val-Gly-Arg-*p*-nitroanilide solution, 10 μL of the test compound solution, and the plate allowed to thermally equilibrate at 37 °C in a molecular devices plate reader for >15 min. Reactions were initiated by the addition of a 10 μL aliquot of enzyme and the absorbance increase at 405 nm is recorded for 15 min. Data corresponding to less than 10% of the total substrate hydrolysis were used in the calculations. The ratio of rate of change in absorbance as a function of time for a sample containing no test compound is divided by the velocity of a sample containing test compound, and is plotted as a function of test compound concentration. The data are fit into a linear regression, and the value of the slope of the line calculated. The inverse of the slope is the experimentally determined K_i value.