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THROMBIN INHIBITORS BASED ON A PROPARGYLGLYCINE TEMPLATE

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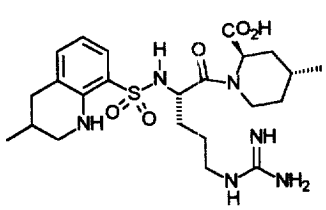
Abstract: A series of novel arylsulfonylpropargylglycinamide derivatives was investigated as thrombin inhibitors in which the SAR was focused on substituents at the acetylenic terminus. Several compounds in this series were identified as potent thrombin inhibitors (Ki up to 5 nM) that are highly selective over trypsin and other serine proteases as well.

Because thrombin plays a pivotal role in the pathogenic thrombosis, its inhibition has been a major target for development of useful antithrombotic therapeutics for patients with diseases such as myocardial infarction, unstable angina, and deep vein thrombosis. While numerous small molecule inhibitors of thrombin have been discovered,[1] argatroban (Novastan, **1**) is represented as the most advanced agent.[2] However, the utility of this agent is limited due to its poor oral bioavailability, which has inspired developing various classes of dipeptide-based thrombin inhibitors with improved pharmacokinetic properties. Compounds **2** (LB30057)[3] and **3** (UK-156,406)[4] have recently been identified as orally active thrombin inhibitors and serve as the prototypical examples in this class.

As a part of our continuing program to discover novel thrombin inhibitors based on a dipeptide motif, we have investigated a series of compounds constructed on a propargylglycine template. Our target compounds are exemplified by the glycine derivatives **4-22**, that incorporate appropriate substituents designed to be recognized by thrombin. The *N,N*-cyclopentylmethylamidyl group was adopted as the C-terminal substituent based on our previous structure-activity relationship information on compound **2** and a related series of thrombin inhibitors in which this nonchiral moiety was optimal for binding in the P-pocket of thrombin.[3,5] We envisioned that the rigid propargyl side chain could be well tolerated by the S1 specificity pocket of thrombin if incorporated with elements capable of interacting with the active site Asp189. Given that the poor oral



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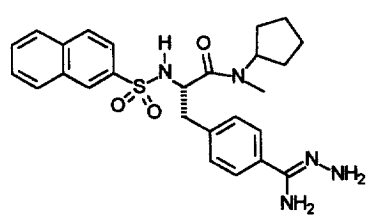
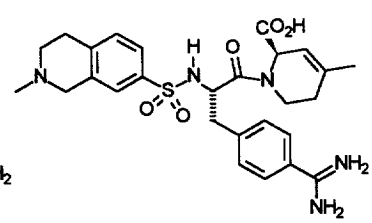


2



3

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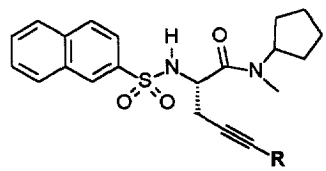
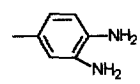
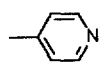
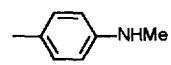
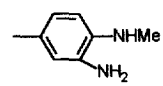
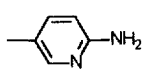
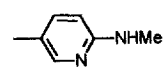
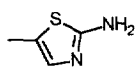
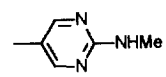
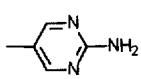
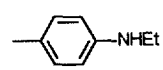
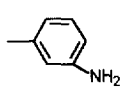
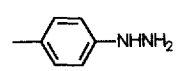
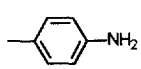
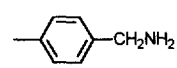
bioavailability of most thrombin inhibitors is in part associated their basic P1 functionalities such as alkylguanidine and benzamidine,[6] we chose to avoid such basic moieties as the acetylenic substituents. Instead we decided to explore the activity of a variety of neutral or mildly basic moieties. Herein we describe the basic SAR study of this novel class, a study that led to identification of several compounds as potent and selective thrombin inhibitors.

As shown in Table 1, the present study commenced with the pyridyl and imidazolyl substituents (**4-6**) because of their structural simplicity and mild basicity. These heterocycles exhibited modest inhibitory activity against thrombin at best. Several aminoaryl moieties (**7-11**) then were chosen in the hope that their amino group might serve as a hydrogen-bonding mediator, thereby causing the desired interaction with Asp189.[7] Of these substituents, the *p*-aminophenyl (**11**) was most active, displaying a 5-fold higher activity compared to the

Table 1. Thrombin[a] and trypsin[b] inhibitory activities of aryl-substituted propargylglycinamides **4-19**.



Compd	R	Ki (μM)	Compd	R	Ki (μM)
4		2.5	**12**		0.22
5		>20	**13**		0.019 37 (trypsin)
6		3.0	**14**		0.040 32 (trypsin)
7		1.5	**15**		0.30
8		4.5	**16**		3.5
9		>20	**17**		49.0
10		2.2	**18**		1.5
11		0.29	**19**		3.4

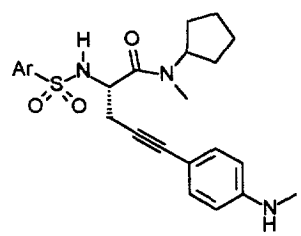
[a] human thrombin [b] bovine trypsin

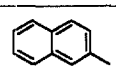
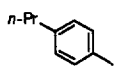
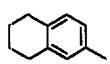
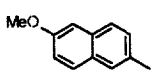
aminopyridyl (7) despite its lower basicity. These results may be compared with the recently reported tripeptidic thrombin inhibitors incorporating aminoaryl residues at P1 in which the aminopyridyl group is superior to the *p*-aminophenyl substituent.[7] The phenylenediamine **12** showed activity only comparable to that of compound **11**.

Encouraged by the sub-micromolar activity observed with compound **11**, our attention was moved to variation of its amino group. To our surprise, the addition of an *N*-methyl group, as in compound **13**, resulted in a 15-fold enhancement in potency with a Ki of 19 nM. A consistent *N*-methyl effect was observed with compounds **14-16**: these derivatives exhibited all markedly enhanced activity in comparison to the parent primary amines. In contrast, the ethyl derivative **17** afforded a severe drop in activity compared to **11**. The benzhydrazine and the benzylamine analogs **18** and **19** also produced significantly diminished potency, reflecting that the *N*-methyl-*p*-aniline group may be an optimal substituent in this series. X-ray crystallographic analysis of compound **13** bound to thrombin demonstrated a novel binding interaction with the specificity pocket. The aniline N-H was shown to be hydrogen-bonded to one of the carboxylic oxygens of Asp189 with its methyl group fitting in the hydrophobic cavity formed by Gly226, Tyr228, Val213, and Ala190 (data not shown).[8] This data also implies that the *N*-ethyl substituent is too bulky to be tolerated. In addition to their substantial activity for thrombin inhibition, both compounds **13** and **14** displayed remarkable selectivity against trypsin, a prototypical thrombin-like serine protease.

The finding of potent activity in compound **13** prompted us to investigate other sulfonamide aryl moieties because we have observed potency improvement for compound **2** and related compounds by replacing

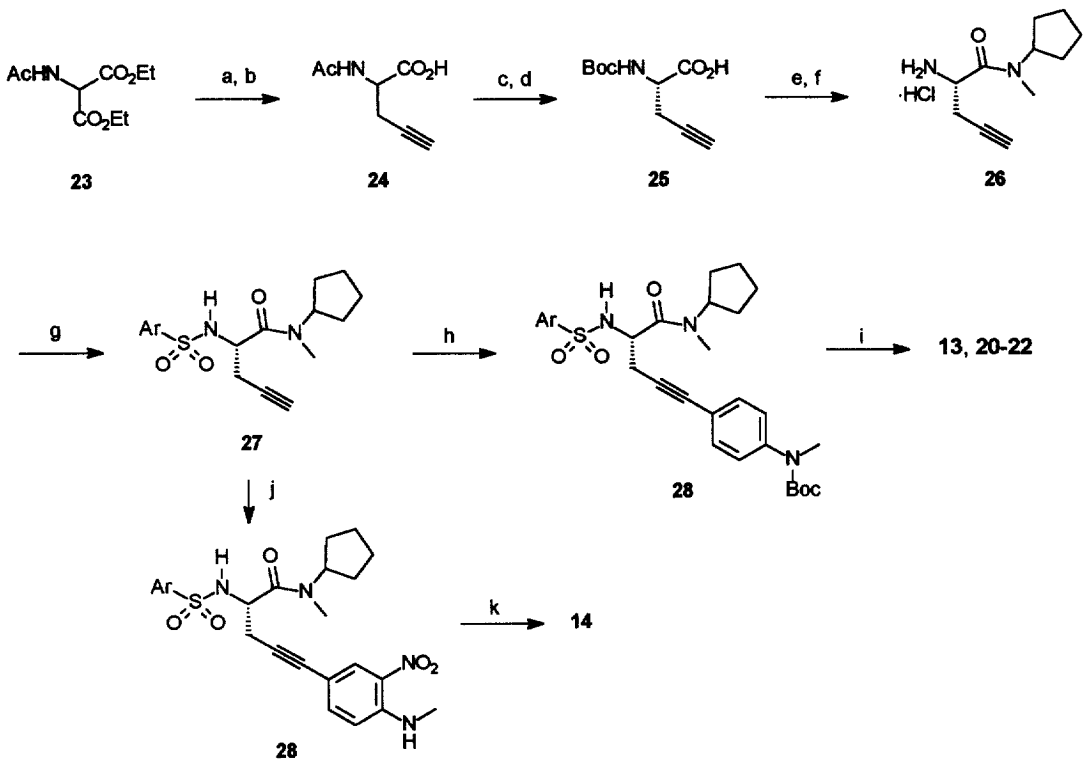
Table 2. Activities of aniline-substituted propargylglycinamides against thrombin and other serine proteases.



Compd	Ar	Thrombin	Trypsin	Plasmin	FXa	t-PA
		Ki (μM)				
13		0.019	37	>200	8.7	>200
20		0.008	>30	>200	6.6	190
21		0.005	>30	>200	1.9	200
22		0.027	>30	>200	14	>200

the naphthyl group with other aryl moieties.[9] Some readily available aryl replacements that were superior in the previous SAR were chosen. While 6-methoxy-2-naphthyl group (**22**) exhibited similar potency, the 4-propylphenyl and 2-tetrahydronaphthyl groups (**20**, **21**) demonstrated potency enhancement as compared to **13**. Interestingly, despite nanomolar Ki's for thrombin inhibition, these compounds were devoid of appreciable inhibitory activity against bovine trypsin and human serine proteases, plasmin, factor Xa, and tissue-type plasminogen activator (Table 2).

Scheme 1



(a) propargyl bromide, NaH, DMF, 95%; (b) NaOH (*1 equiv*), H_2O/EtOH, reflux, 90%; (c) Hog acylase, H_2O, pH 6.5, 37 °C, 3 days; (d) Boc_2O, NaOH, H_2O/dioxane; (e) *N,N*-cyclopentylmethylamine-HCl, NMM, EDC, Hobt, DMF, 52% from **24**; (f) AcCl, MeOH; (g) arylsulfonyl chlorides, NMM, DMF, 88-94%; (h) $Pd(Ph_3P)Cl_2$, CuI, Et_3N, *N*-Boc-*N*-Me-4-iodoaniline, CH_3CN, rt, 5h, 72-88%; (i) 50% TFA in CH_2Cl_2, 12h, 65-78%; (j) CuI, $Pd(Ph_3P)Cl_2$, Et_3N, *N*-Me-2-nitro-4-iodoaniline, CH_3CN, 77%; (k) $SnCl_2$, MeOH, reflux, 77%.

The synthesis of potent thrombin inhibitors of this series is outlined in Scheme 1. The requisite amino acid template, *N*-Boc-L-propargylglycine (**25**), was prepared in four steps from diethyl acetylaminomalonate (**23**) and propargyl bromide essentially according to the literature procedure.[10,11] Standard amino acid coupling of compound **25** with *N,N*-cyclopentylmethylamine, subsequent deprotection of the N-terminal Boc group, and sulfonylation with arylsulfonyl chlorides[12] led to compounds **27**. These intermediates smoothly underwent

palladium-catalyzed acetylenic coupling[13] with Boc-protected *N*-methyl-4-iodoaniline at room temperature to give compounds of the general structure **28** in good yields. The Boc group was efficiently removed by treatment with 50% trifluoroacetic acid in methylenechloride to provide the target compounds **13** and **20-21**. The phenelenediamine compound **14** could be obtained by an acetylenic coupling with *N*-methyl-2-nitro-4-iodonitroaniline,[14] followed by reduction of the nitro group of the resulting adduct **29**. Other compounds listed in Table 1 were prepared similarly from the corresponding aryl halides[15] and the versatile intermediate **27**.

In conclusion, we have studied a series of arylsulfonylpropargylglycinamide derivatives by screening a variety of substituents at the acetylenic terminus and identified some potent thrombin inhibitors. *N*-Methyl-*p*-aniline group was of highest interest as nanomolar Ki values for thrombin were achieved with compounds **13** and **20-22**. Also noteworthy is the excellent selectivity of these compounds for thrombin versus trypsin and other serine proteases. Furthermore, pharmacokinetic evaluation of the best compound **21** revealed its oral absorption behavior (C_{max} = 1.8 μM, T_{max} = 45 min, AUC = 4.6 μM.h, 30 mg/kg in rats, n = 2).[16] The impressive results reported here suggest that this type of aniline-based acetylenic scaffold may compare favorably with the conventional P1 amino acid elements of dipeptide-based thrombin inhibitors such as arginine and amidinophenylalanine. Further SAR studies of this series are currently in progress to establish the range of substituents. These results will be reported in due course.

Acknowledgment. The authors thank Dr. Y. S. Oh for helpful discussions during this work and S. H. Lee for absorption test with compound **21**.

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8. Details of this data will be described in a later publication.
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11. In the saponification/decarboxylation step (step b), use of one equivalent of base was found be effective for both high yield and high reproducibility.
12. Tetrahydronaphthylsulfonyl chloride was prepared by treatment of tetrahydronaphthalene with chlorosulfonic acid.[9a]
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15. Examples: *N*-trityl-4-iodoimidazole (**6**), 2-amino-5-bromopyridine (**7**), *N*-Boc-2-amino-5-bromothiazole (**8**), *N*-Boc-4-iodoaniline (**11**), 2-nitro-4-iodoaniline (**12**), *N*-methyl-2-amino-5-bromopyridine (**15**), *N*-Boc-4-bromobenzylamine (**19**).
16. Details of experimental protocols are described in ref 9c.