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Noncovalent Thrombin Inhibitors Incorporating an Imidazolylethynyl P1

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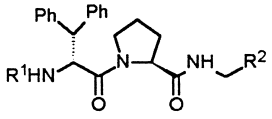
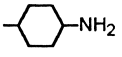
Dedicated to Professor David F. Wiemer on the Occasion of His 50th Birthday

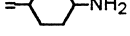
Abstract—A series of noncovalent tripeptidic thrombin inhibitors incorporating a unidazolylethynyl moiety at P1 was investigated. A number of compounds of this series were highly potent and selective versus trypsin, and several compounds demonstrated good oral absorption in rats (F = 58% for compound **19**).

The development of an orally active thrombin inhibitor represents a still elusive goal. Such a drug would be effective and safe for the prophylaxis of venous and arterial thrombosis. While numerous small molecule thrombin inhibitors have been discovered,[1] much recent attention has been devoted to noncovalent tripeptides that lack an electrophilic serine trap.[2–4] Owing to their fast-binding nature, the noncovalent inhibitors are more efficacious and thus have a better therapeutic index than covalent inhibitors.[1b,4c,5] However, only a few compounds of this class are reported to have oral bioavailability. Unless their log P values are improved by incorporation of highly lipophilic groups,[6] thrombin inhibitors with strongly basic P1 elements are likely to have poor oral bioavailability. Compounds in this class are typified by the Merck products **1**–**3**; the cyclohexylamine compound **1**[3b] and its derivatives lack oral bioavailability while the less basic aminopyridine analogue **3**[3c] is highly bioavailable in dogs.

We recently investigated a series of arylsulfonyl-propargylglycinamide thrombin inhibitors possessing a variety of weakly basic P1 probes at the ethynyl terminus, and identified a number of potent and orally bioavailable thrombin inhibitors (e.g. **4**, $K_i = 5$ nM).[7] The conformationally rigid ethynyl tether was best tolerated by the S1 specificity pocket of thrombin when it was substituted by an *N*-methylaniline moiety whereas the corresponding alkyl and vinyl analogues[8] were poorly tolerated. As an extension of this work, we have incorporated this novel ethynyl P1 into the D-Phe-Pro scaffold and found a new class of noncovalent thrombin inhibitors. In this paper, we describe the results of this study.



1: R^1 = H, R^2 = 

2: R^1 = SO_2CH_2Ph, R^2 = 

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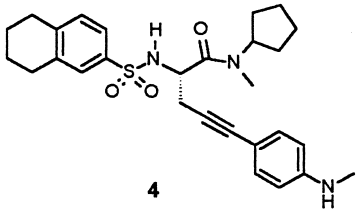

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With a readily available *N*-Boc-D-Phe-Pro-NH-CH_2-C≡CH template, we explored several heterocyclic substituents at the acetylenic terminus including imidazole, pyrazole, pyridine and 2-aminopyridine, chosen on the basis of size, basicity, and potential capability of interacting with Asp189 in the S1 active site of thrombin (Table 1). The imidazole **5**, as its acid salt, appeared to be the most soluble compound in this series, and to

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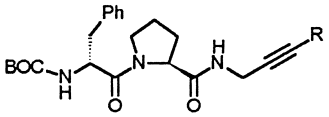
Table 1. Thrombin inhibitory activity for compounds **5**–**9**



Compounds	R	Thrombin K_i (μM)
5	Imidazol-4-yl	1.2
6	3(N)-Me-imidazol-4-yl	5.5
7	Pyrazol-4-yl	5.4
8	Pyrid-4-yl	>20
9	2-Aminopyrid-5-yl	>20

exhibit the greatest thrombin affinity. The aminopyridine P1 substituent (compound **9**) appeared to be too large to be tolerated by the S1 pocket, while introduction of a methyl group on the imidazole nitrogen of **5** closer to the ethynyl substituent (compound **6**) was detrimental.

In view of reports that replacement of D-Phe at P3 with the unique amino acid D-diphenylalanine resulted in a dramatic potency increase in the peptidic thrombin inhibitors,[3b,9] we anticipated that the potency of compound **5** might be significantly improved by utilizing this amino acid. Indeed, the diphenylalanine compound **10** exhibited a 60-fold potency increase with a K_i of 24 nM and good selectivity against trypsin as well. Remarkably, this compound was well absorbed when administered orally in rats (C_{max} = 14.6 μM, T_{max} = 60 min, AUC = 29 μM·h, 30 mg/kg, n = 3) whereas the des-Boc analogue **11**[10] was significantly less absorbed.

Compound **10** however exhibited only moderate anticoagulant activity when tested for its ability to prolong activated partial thromboplastin time (aPTT) in human plasma, suggesting that higher thrombin inhibitory potency would be necessary for in vivo effectiveness. Incorporation of highly lipophilic amino acid D-dicyclohexylalanine[6] gave analogue **12** that maintained comparable potency but exhibited a significant loss of anticoagulant activity. In view of a recent report that the benzylsulfonyl group of **2** occupied a new binding pocket in thrombin, thereby leading to greatly increased affinity,[3a] we prepared compound **13**.[10] It displayed a 10-fold enhanced potency and excellent anticoagulant activity in the clotting assay, but the benzylsulfonyl group proved detrimental to oral absorption.

X-ray crystallographic studies on the complex of **13** with thrombin showed that the inhibitor was bound in the active site in a fashion very similar to that of the reported compound **2** (Fig. 1). The diphenyl and proline rings spanned the distal and proximal hydrophobic pockets, respectively, while the N–H of the imidazolylethynyl side chain hydrogen-bonded to the carboxylic acid of Asp 189 of the specificity pocket (2.77 Å). There was also evidence for a hydrogen bond with a water molecule involving the N–H of the alternate imidazole tautomer. One of the sulfonyl oxygens was hydrogen-bonded with the N–H of Gly 219 (3.27 Å). Although the phenyl ring of the benzylsulfonyl group appeared to

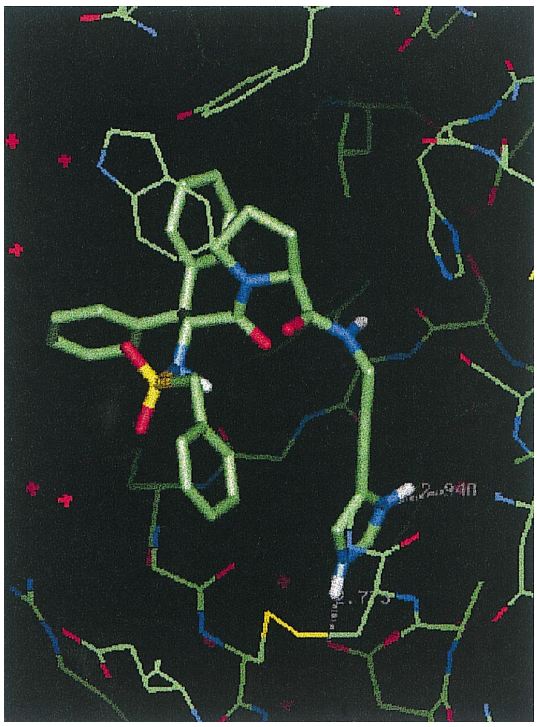


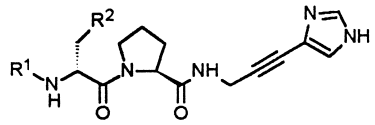
Figure 1. X-ray structure of compound **13** bound to thrombin.

be in close contact with the imidazole ring, it did not show well-defined electron density, indicating a very weak contribution of the phenyl ring to the enzyme affinity.

Consistent with the X-ray crystallographic analysis, the methanesulfonyl compound **14** retained potency with a K_i of 2.6 nM. Interestingly, the related sulfamoyl compound **15** exhibited even better potency with a subnanomolar K_i. The potency of these sulfonyl compounds translated well into good anticoagulant activity (see Table 2) that compares favorably with that of the efficacious oral thrombin inhibitor LB30057,[11] previously discovered in our laboratories. However, both compounds **14** and **15**, like compound **13**, were poorly absorbed. The acetyl and the carboxymethyl compounds **16**–**18** were to some extent orally bioavailable in rats (C_{max}, 1.6–5 μM, 30 mg/kg, po), but displayed limited potency.

Molecular modeling of the carbamate **10** revealed that the Boc *t*-butyl moiety was exposed to solvent and did not interact with the thrombin active site. We therefore prepared the methyl carbamate **19**, which indeed was found to be equipotent to **10**. In particular, the plasma level of **19** was significantly high after oral administration in rats (C_{max} = 23.6 μM, T_{max} = 60 min, AUC = 46 μM·h, 30 mg/kg, po, n = 3; Cl = 12.8 mL/min/kg $t_{1/2}$ = 40 min, AUC = 26 μM·h, 10 mg/kg, iv, n = 3; F = 58%). Compound **19** also exhibited good anticoagulant activity, although still less than desired. By contrast, and similar to the results obtained with **12**, the equipotent dicyclohexyl analogue **20** was relatively inactive in the clotting assay. This result is in accord with Merck's findings.[6]

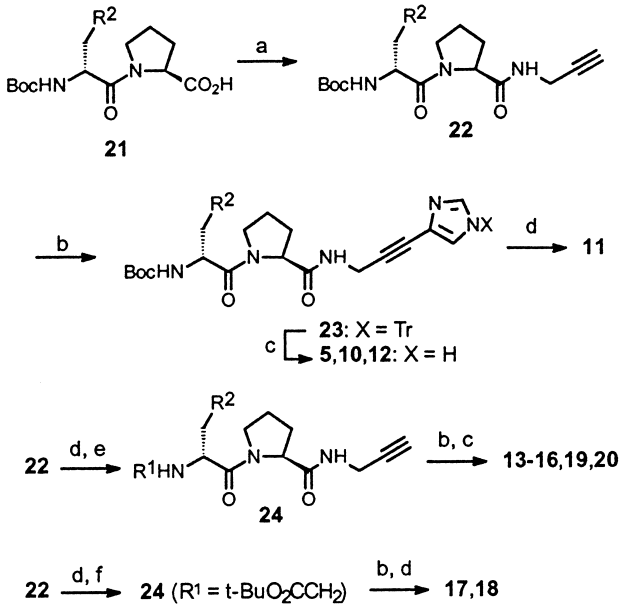
Table 2. Thrombin and trypsin inhibitory activities and coagulation parameter for compounds **10**–**20**



Compds	R^1	R^2	Thrombin[a] K_i (nM)	Trypsin[b] K_i (nM)	2×aPTT[c] (μg/mL)
10	Boc	Diphenyl	24	50	6.0
11	H	Diphenyl	12	25	3.4
12	Boc	Dicyclohexyl	29	30	>20
13	$BnS(O)_2$	Diphenyl	3.0	24	1.0
14	$MeSO_2$	Diphenyl	2.6	14	1.2
15	$NH_2S(O)_2$	Diphenyl	0.8	18	0.55
16	Ac	Diphenyl	208	ND	ND
17	HO_2CCH_2	Diphenyl	44	47	ND
18	HO_2CCH_2	Cyclohexyl	480	ND	ND
19	MeO(CO)	Diphenyl	14	24	1.6
20	MeO(CO)	Dicyclohexyl	15	30	>10
LB30057			0.4	33	0.89

[a]Human thrombin.
[b]Bovine trypsin.
[c]Concentration of inhibitor in human plasma required to double the activated partial thromboplastin time (aPTT).

The synthesis of the imidazole thrombin inhibitors in Table 1 and 2 is outlined in Scheme 1. The propargyl intermediates **22**, prepared by amide coupling of dipeptides of the general structure **21**[6,12] with propargylamine, underwent Pd-catalyzed acetylenic coupling with *N*-trityl-4-iodoimidazole[13] to give **23** in good yields. The trityl group was efficiently removed by treatment with TFA and triisopropylsilane. Compounds **7**–**9** were also prepared in this manner from **22** and the corresponding heterocyclic bromides. Compound **6** could be prepared by *N*-methylation (Me_3OBF_4) of **23** (R^2 = phenyl), followed by trityl deprotection.



Scheme 1. (a) Propargylamine, NMM, EDC, HOBT, quant.; (b) $Pd(Ph_3P)_4$, CuI, Et_3N, *N*-trityl-4-iodoimidazole, CH_3CN, rt, 15 h, 60–90%; (c) TFA, *i*-Pr_3SiH, CH_2Cl_2, 0 °C, 2 h; (d) TFA, neat, rt; (e) R^1-Cl, Et_3N, CH_2Cl_2, 52–88%; (f) *t*-BuO_2CCH_2Br, Et_3N, CH_3CN, rt, 24 h, 70%.

In summary, we have investigated a series of non-covalent tripeptidic thrombin inhibitors that incorporate an imidazolylethynyl P1. With benefit from the weakly basic P1 motif, a number of compounds were prepared that demonstrated good oral absorption in rats. The most interesting compound in this series was the carbamate **19**, that showed both good oral bioavailability and good anticoagulant activity. However, utility of this compound was limited by its moderate antithrombotic activity when evaluated in the rat venous thrombosis model. Future communications will report details of our continuing efforts to develop more efficacious oral thrombin inhibitors.

Acknowledgements

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