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Active Site-directed Thrombin Inhibitors—II. Studies Related to Arginine/Guanidine Bioisosteres

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Abstract—A series of *N*-arylsulfonylarginine amides was synthesized wherein the guanidine or arginine moiety was isosterically replaced by a number of heterocyclic functionalities. These compounds were evaluated as potential active-site inhibitors of thrombin. Bisamidines **11a–n** showed a similar SAR to that of simple arginine compounds. The *ex vivo* clotting time measurement of **11d** after ip dosing showed prolongation of clotting time in rats.

Introduction

Thrombosis can be regarded as a pathological condition where improper regulation of the hemostatic mechanism results in the formation of intravascular thrombi which may lead to tissue damage or cell death due to inadequate blood flow. This condition is a major cause of mortality and morbidity in humans. The crucial importance of the coagulation enzyme thrombin in the pathogenesis of thrombosis has become clearer due to the availability of inhibitors of this enzyme and, therefore, the development of thrombin inhibitors as therapeutic agents has become a major focus.[1] Thrombin plays a major role in hemostasis and thrombosis. The serine protease activity of thrombin is required for the cleavage of fibrinogin to fibrin polymer and also for platelet activation. Traditional antithrombotic agents like coumarins and heparin invariably suffer from adverse side effects such as thrombocytopenia, osteoporosis and triglyceridemia. Their slow onset and reversal of action as well as bleeding complications and an unpredictable therapeutic safety margin are the major clinical problems associated with the use of these agents. Thus, the development of agents to inhibit thrombin generation and activity in prethrombotic events could greatly aid in lowering the incidence of thrombus-induced myocardial and cerebral ischemia.

The evolution of specific synthetic inhibitors was partly due to the consideration of natural substrates, such as fibrinogen, cleavage sites, amino acid sequences and also due to the availability of the naturally-occurring inhibitors such as hirudin. A number of thrombin active-site directed inhibitors that are reversible, irreversible as well as slow tight binding reversible have appeared in the literature.[2] Some of these agents have shown efficacy in animal models of thrombosis. Argatroban, MD 805, is the most notable thrombin inhibitor that has advanced to clinical trials and has been approved as an iv agent.[3]

It is reasonable to consider that for therapeutic use of low molecular weight inhibitors, the competitive mode of action is more desirable since no stable derivatives of the enzyme can occur. Other important aspects of such a drug are toxicity, *in vivo* half life and oral bioavailability. With these considerations, we started exploring a number of approaches to design active site inhibitors of the enzyme thrombin. There exist some suggestions in the literature that the lack of oral bioavailabity and increased toxicity might be attributed to highly basic functionalities such as guanidine.[4] Introduction of an acidic moiety as an internal neutralizing group has been explored by the Mitsubishi group.[5] In this article, we describe the preparation and biological evaluation of a series of *N*-arylsulfonylarginine amides as thrombin inhibitors wherein several novel heterocycles or functionalities with reduced basicity were introduced as bioisosteric replacements of the guanidine/arginine moiety.

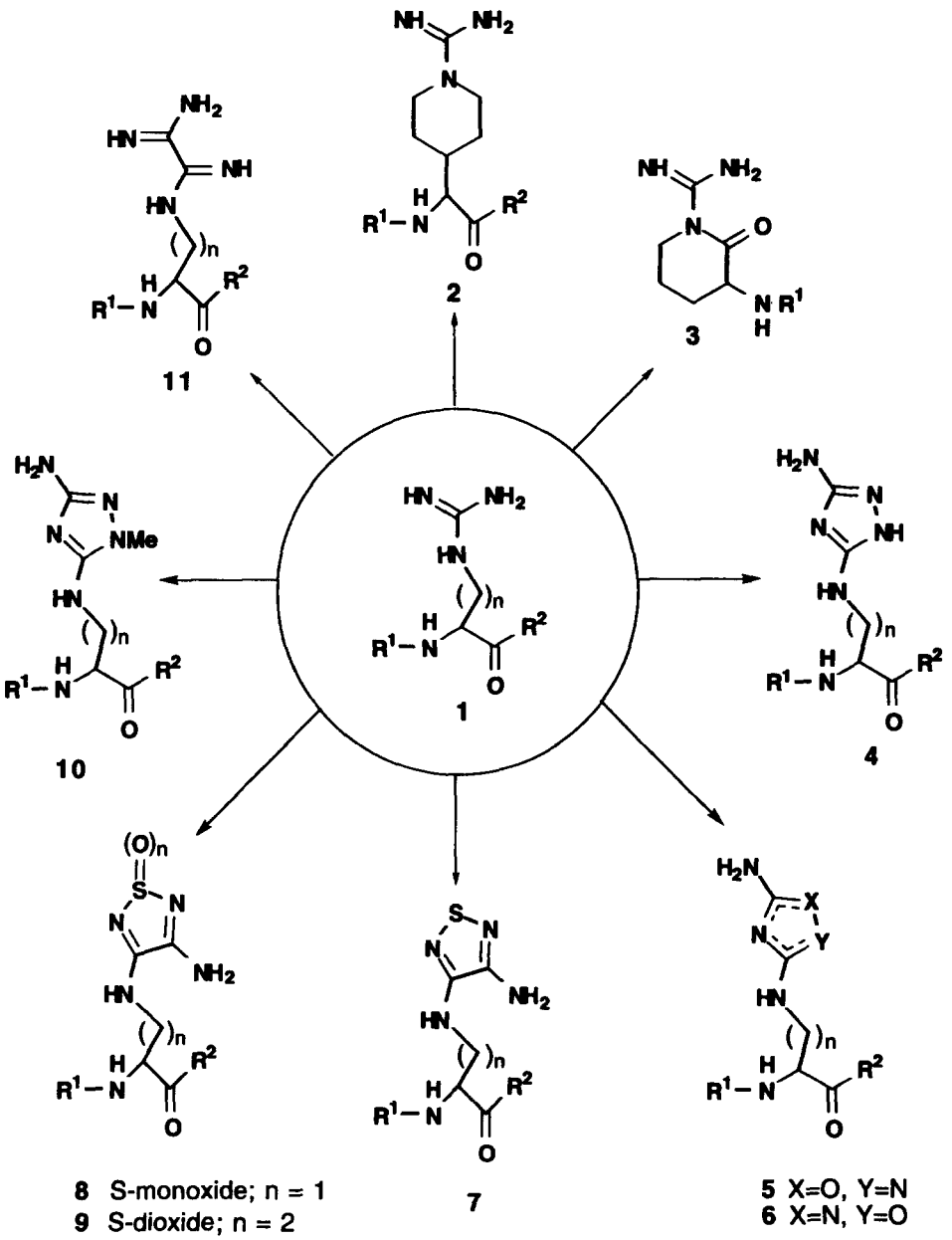
Chemistry

The different arginine/guanidine surrogates prepared as part of this study are listed in Scheme 1. A two-step procedure was employed for the general preparation of various *N*-arylsulfonylcarboxamide derivatives employed in this study. Treatment of the starting amino acid with an appropriate N-arylsulfonyl chloride provided the corresponding sulfonamide after workup. DPPA-mediated coupling of the acid with the desired amine furnished the key *N*-arylsulfonamide derivative in good yield. All of the starting amino acids discussed herein were readily obtained from either commercial sources

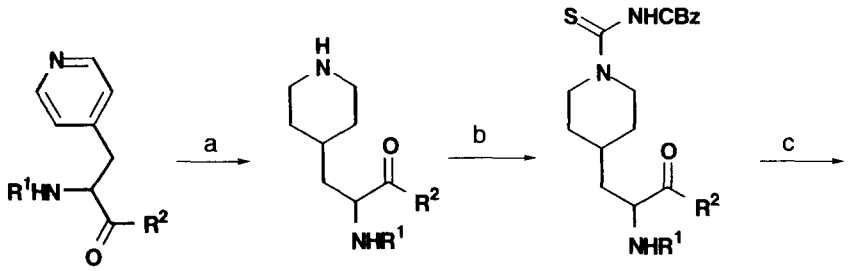
or prepared by following known literature procedures.[5] The syntheses of racemic *N*-amidinopiperazinyl derivatives **2a** and **2b** are depicted in Scheme 2.

Thus, catalytic hydrogenation of **12a** and **12b** followed by stepwise guanidination employing addition of *N*-CBz isothiocyanate, activation of the thiourea with methyl iodide and subsequent treatment with ammonia and hydrogenation produced the free guanidines which were converted to their HCl salts, **2a** and **2b**, respectively. The cyclized arginine compounds **3a** and **3b** were prepared from the previously reported[6] lactam **16** as described in Scheme 3. The general synthetic sequence involved in the preparation of a series of bis-amidines or bis-guanidines is outlined in Scheme 4. Thus, catalytic hydrogenation of **19a–n** in the presence of an acid produced the amines as HCl salts. Reaction of amines **20a–n** with 3-amino-4-methoxy-1,2,5-thiadiazole-S-oxide in refluxing methanol for a period of 1–5 h gave the thiadiazole S-oxide intermediates **8a–n**. Hydrolysis under acidic conditions furnished bisamidines **11a–n** as their HCl salts.[7]

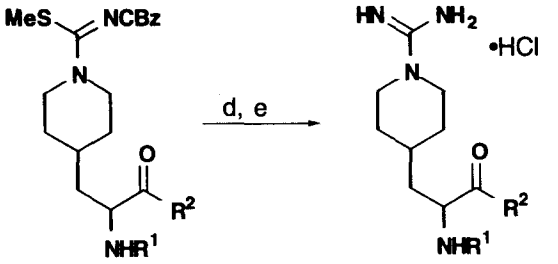
Intermediate amine **20d** was smoothly transformed into a number of different heterocyclic nuclei. As such, reaction of **20d** with dimethyl *N*-cyanodithioiminocarbonate in ethanol and 10% aqueous NaOH afforded *N*-cyano-S-methylthioguanidine **21**. Conversions of **21** to triazoles **4** and **10** and oxadiazoles **5** and **6** were carried out by reacting **21** with the appropriate hydrazines and hydroxylamines (Scheme 5). The regioisomeric oxadiazoles **5** and **6** were evaluated as a mixture. Similarly, treatment of amine **20d** with 3-



Scheme 1.



12a-b 13a-b 14a-b

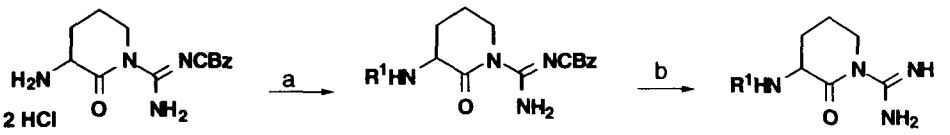


15a-b

2a R^1=dansyl, R^2=piperidine
2b R^1=tosyl, R^2=piperidine

(a) 10% Pd/C, H_2, MeOH; (b) CBzHNCS, THF; (c) MeI, acetone; (d) NH_3, THF; (e) HCl, EtOH.

Scheme 2.



16 17a-b

3a R^1=dansyl
3b R^1=tosyl

(a) R^1Cl, NMM, DMF; (b) 1 N HCl, 10% Pd/C, H_2, EtOAc.

Scheme 3.

amino-4-methoxy-1,2,5-thiadiazole-S,S-dioxide[7] in refluxing methanol gave the desired product **9** in good yield (Scheme 6). Finally, reaction of bis-amidine **11d** with *N,N'*-thiobisphthalimide produced thiadiazole **7** without incident.[8]

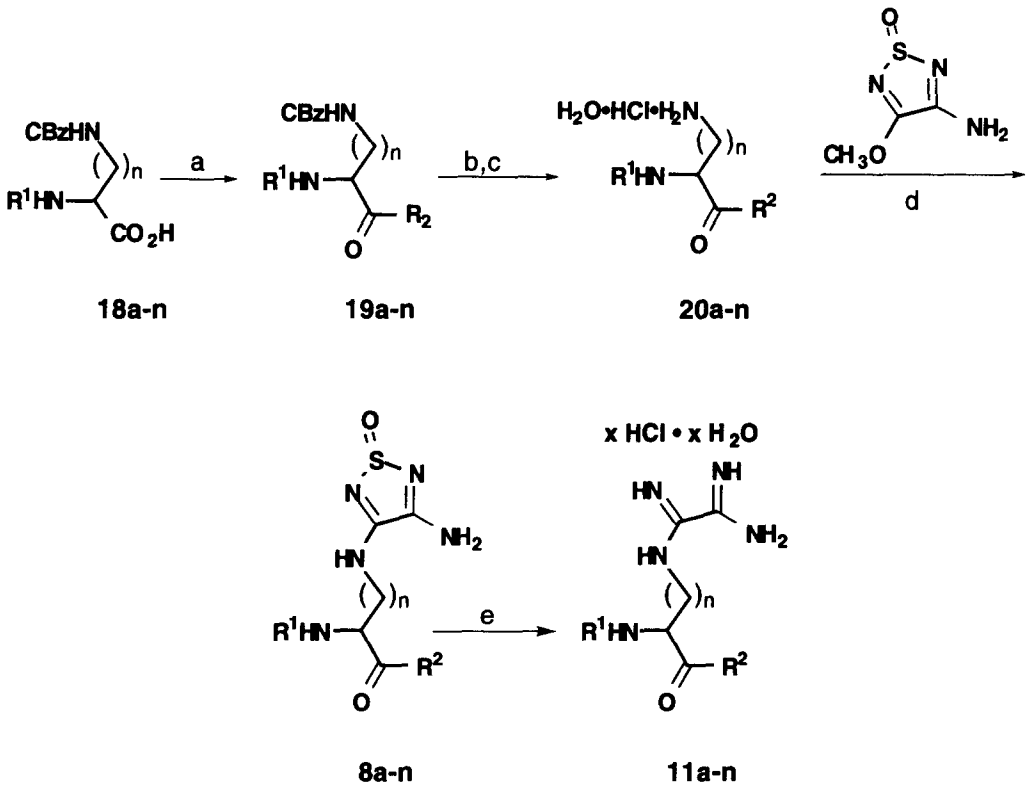
Biological Evaluation

The target compounds prepared were evaluated as inhibitors of thrombin catalytic activity using the chromogenic substrate s-2238. The effective concentration (EC_{50}) for thrombin inhibitory activity was determined from dose–response curves. The K_i values were obtained from standard Dixon plots. The effect of drugs on the clotting time was determined in human platelet-poor plasma (*in vitro*) and in rats (*ex vivo*). The concentration of drug required to double the time to clot formation, as compared to drug-free control, was measured and presented as DCT.

The measure of oral efficacy of the target compounds was determined by *ex vivo* clotting time measurements from oral dosing of the inhibitors in male Sprague–Dawley rats (fasted) followed by drawing blood samples. The duration of action in the rat was determined by measuring the *ex vivo* clotting times after ip dosing at various time points.[9]

Results and Discussion

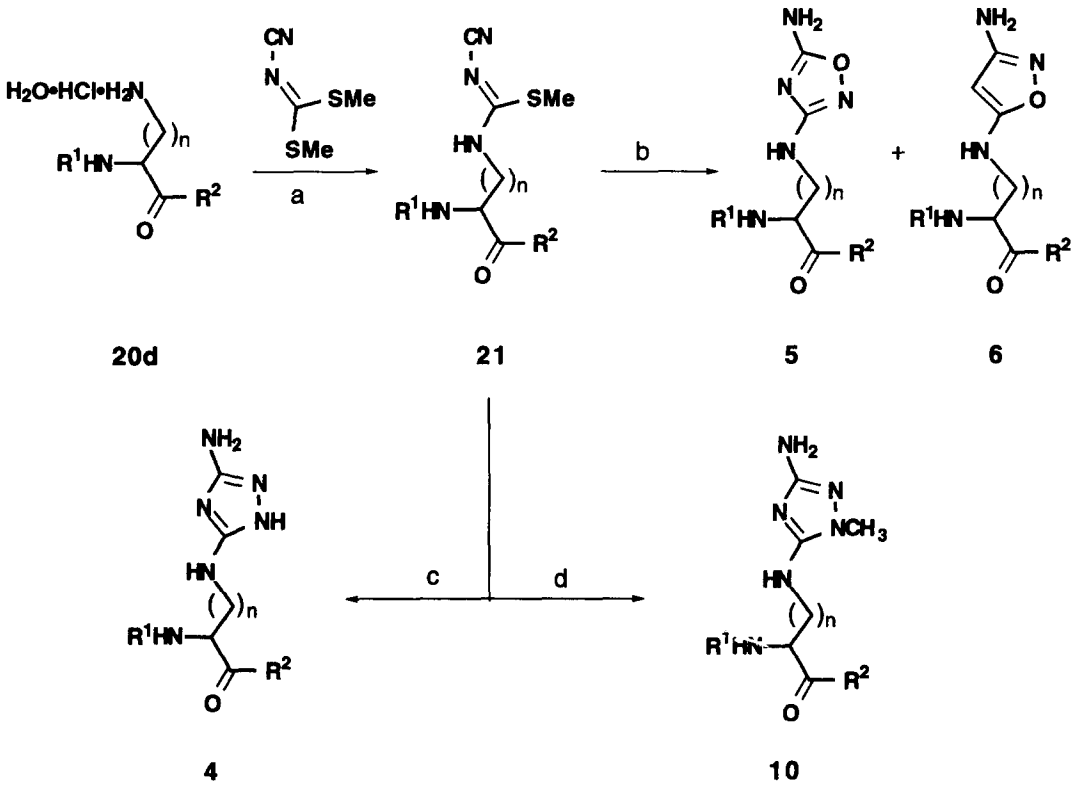
Our approach to the design of thrombin inhibitors focused mainly on replacing the guanidine/arginine moiety in a series of *N*-arylsulfonyl arginine amides with a variety of heterocycles or other functionalities of suitable basicity. It is now evident from X-ray crystal structures that inhibitor-bound thrombin complexes which contain guanidine-like moieties interact with Asp[189] in the enzyme to form a salt bridge.[10] Such key interactions have also been taken into consideration



For R^1, R^2 and n see Table 2.

(a) DPPA, DMF, TEA, R^2H; (b) 5% HCO_2H/MeOH, 10% Pd/C, rt; (c) HCl/MeOH/CH_2Cl_2; (d) MeOH, TEA; (e) conc. HCl, MeOH.

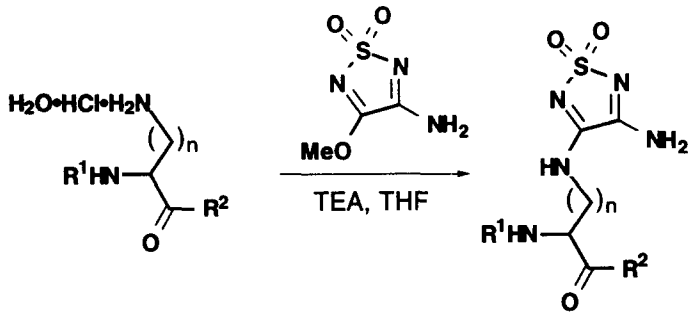
Scheme 4.



where R^1 = dansyl, R^2 = 4-ethylpiperidine, n = 3.

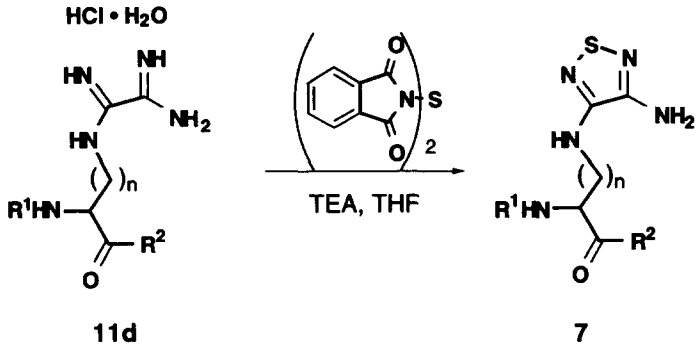
(a) NH_2NH_2, TEA, EtOH; (b) NH_2NHCH_3, TEA, EtOH; (c) TEA, EtOH; (d) NH_2OH·HCl, TEA, EtOH.

Scheme 5.



where R^1 = dansyl, R^2 = 4-ethylpiperidine, n = 3.

Scheme 6.



where R^1 = dansyl, R^2 = 4-ethylpiperidine, n = 3.

Scheme 7.

during the design process. Table 1 illustrates representative examples of different arginine/guanidine isosteres prepared as part of this study and their thrombin inhibitory activity. In an attempt to elicit maximum response between different series, we selected the dansyl and piperidine functionalities as the substitution partners. In general, most of the different isosteric replacements resulted in compounds with thrombin inhibitory potencies in the range of 1–50 μM. The *N*-amidino heterocycles **3a** and **3b** represent the simple cyclized versions of **1a** and **1b**, respectively. The internal acylation in lactam **3** was expected to modulate the basicity of the guanidine moiety. The dansyl analog **3a** displayed a K_i value of 3 μM, while

Table 1. Thrombin inhibitory activities of representative guanidine/arginine bioisosteres of **1** in chromogenic and clotting time assays

Compound	‡R^1	R^2	n	K_i (μM)	†DCT (μM)
1a	dansyl	piperidine	3	0.90	–
1b	tosyl	piperidine	3	14	–
2a	dansyl	piperidine	–	20	–
2b	tosyl	piperidine	–	50	–
3a	dansyl	–	–	3	10
3b	tosyl	–	–	12.5	> 200
4	dansyl	4-ethylpiperidine	3	16	100
*5,6	dansyl	4-ethylpiperidine	3	> 50	> 100
7	dansyl	4-ethylpiperidine	3	0.2	15
8d	dansyl	4-ethylpiperidine	3	15	200
9	dansyl	4-ethylpiperidine	3	1.0	150
10	dansyl	4-ethylpiperidine	3	13	100
11d	dansyl	4-ethylpiperidine	3	0.3	0.92

*Isolated as a mixture.
‡Dansyl = 5-dimethylamino-1-naphthalenylsulfonyl.
‡Tosyl = *p*-methylphenylsulfonyl.
†DCT = concentration of drug required to double the time to clot formation, as compared to drug-free control.

the tosyl counterpart **3b** was much less active. This is comparable to the open chain reference agent reported in the literature.[5a] A similar trend was also observed in the racemic *N*-amidinopiperazine compounds **2a** and **2b**.

A second series of prototypes (**4–10**) were prepared to test the hypothesis that different heterocycles with potentially H-bonding nuclei in them might act as surrogates for guanidine. The aminotriazoles (**4** and **10**), aminoxadiazoles (**5** and **6**) and the aminothiadiazoles (**7**, **8** and **9**) were all found to be less active. This suggests that replacement of guanidine with these heterocycles results in a less favorable binding mode for these inhibitors. Interestingly, thiadiazole **7** and its dioxide **9** showed sub-micromolar binding affinities.

A modified thrombin time was used to monitor the direct inhibition of human thrombin activity in plasma containing samples. Selected compounds from the different series mentioned above were further evaluated in this assay to assess the effective concentration required to double the clotting time (DCT). Although these compounds showed thrombin inhibitory activities against the chromogenic substrate in low micromolar range, they were found to be much less effective in doubling clotting time in human plasma (Table 1).

In a third prototype class, the guanidine group was replaced with a bis-amidine functionality. Thus, the first series of compounds with a two-carbon linker (**11b** and **11i**) was prepared and found to be much less active than their guanidine counterparts. However, the three-carbon homologue (**11c**) displayed a K_i value of 0.33 μM. Thus, a limited number of compounds was synthesized in this series and their inhibitory potencies were evaluated as depicted in Table 2. Increasing or decreasing the methylene linker (**11h**, **11i** and **11k**) resulted in diminished thrombin inhibition. Replacement of the dansyl substituent with tosyl (**11j**) or nasylglycyl (**11m**) residues was also not well tolerated. Replacement of the piperidine nucleus with morpholine (**11e**), perhydroazulene (**11f**) or the open chain glycine ester (**11g**) seemed to be tolerated. In the three-carbon dansyl series (i.e. $n = 3$), however, lipophilic substitution at the 4-position of the piperidine ring increased the inhibitory potency. Thus, 4-methylpiperidide **11c** was found to be 10 times more potent than **11a.** This compound also showed very good selectivity for thrombin over trypsin (40 μM) and plasmin (23 μM). Further increase in lipophilicity as in the case of **11d** only moderately increased the potency. This series of bis-amidines, in general, followed a similar trend in their SAR as did their arginine counterparts.[5a]

The dansyl-Arg-(bis-amidine)-4-ethylpiperidide **11d** represents the lead compound in this series. The biochemical and pharmacological characterization of various *in vitro* and *in vivo* clotting time measurement assays were examined in some detail. Compound **11d** exhibited clean, competitive inhibitory kinetics with no time-dependence to thrombin inhibition. This suggests that **11d** is a reversibile inhibitor. The K_i value for thrombin inhibition averaged 0.3 μM (obtained from Dixon plots). The concentration required to double the clotting time *in vitro* in human platelet-poor plasma was determined to be 0.92 μM ($n = 3$) (Fig. 1). *Ex vivo* clotting time measurements were used to determine the oral activity of **11d**. Thus, when the compound was given orally to rats, no marked change in clotting time was observed suggesting a lack of oral activity. However, ip administration of **11d** significantly improved the prolongation of clotting time. This might indicate poor absorption after oral dosing. Significant prolongation of clotting time was observed at a dose of 20 mg kg^{-1} ip ($n = 2$). Furthermore, the duration studies using *ex vivo* clotting time measurements indicated that **11d** was active at a dose of 20 mg Kg^{-1} ip after 30 min but the activity dropped 50% after 1.5 h (Fig. 2). Similar results were observed for the corresponding guanidine counterpart **1a**.

Table 2. Thrombin inhibitory activities of various bis-amidine bioisosteres in chromogenic and clotting time assays

Compound	R^1	R^2	*n*	K_i (μM)	†DCT (μM)
11a	dansyl	piperidine	3	3.2	–
11b		4-methylpiperidine	2	6	–
11c		4-methylpiperidine	3	0.33	1.0
11d		4-ethylpiperidine	3	0.3	0.92
11e		morpholine	3	2	4
11f		perhydroazulene	3	0.4	1.8
11g		ethyl butylglycinate	3	3	15
11h	tosyl	piperidine	1	insol.	> 300
11i		piperidine	2	80	–
11j		piperidine	3	16	–
11k		piperidine	4	55	150
11l		4-methylpiperidine	3	18	–
11m	*nasylgly	piperidine	2	36	–
11n		piperidine	3	> 50	–

*Nasylgly = 2-naphthalenesulfonylglycyl.
†DCT = concentration of drug required to double the time to clot formation, as compared to drug-free control.

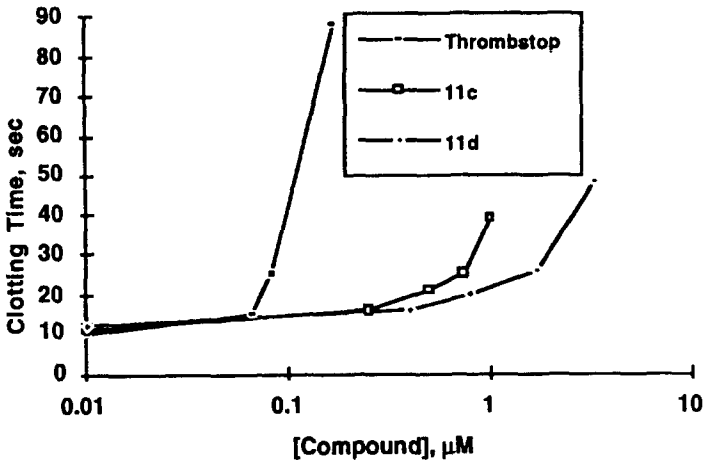


Figure 1. Effects of **11d**, **11c** and Thrombstop (reference agent) on clotting times assayed in human platelet-poor plasma determined *in vitro*.

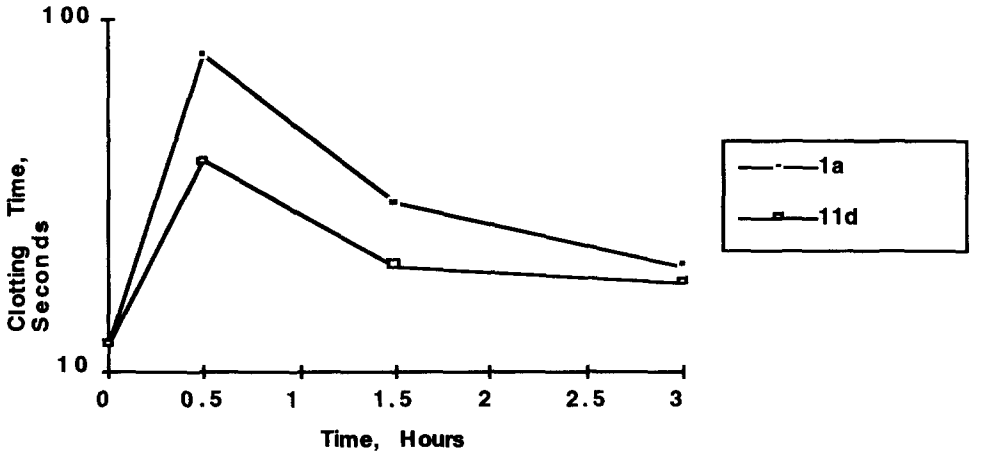


Figure 2. Duration studies. *Ex vivo* clotting time measured at various time points after 20 mg kg $^{-1}$ ip dosing of **11d** and **1a** in rats.

In summary, of the several arginine/guanidine isosteric replacements explored in this study, the bis-amidine showed a similar biological activity and structure–activity relationship profile to the corresponding arginine amides. The *ex vivo* measurement of the doubling of clotting time following po and ip administration of **11d** indicated poor bioavailability and short duration of action. While the bis-amidines showed a similar activity profile to the arginine series, no significant improvement in pharmacological activity was observed to warrant further development of this class of compounds.

Experimental Section

Biology

Enzyme assays for the inhibition of thrombin. The following reagents were used in these assays: thrombin assay buffer: 145 mM NaCl, 5 mM KCl, 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 1 mg mL^{-1} polyethylene glycol (PEG-8000), pH 7.4; 3 mM D-Phe-Pip-Arg-*p*-nitroanilide (s-2238) in H_2O; 3 U mL purified human α-thrombin dissolved in thrombin assay buffer. Inhibitors to be tested were dissolved in H_2O, methanol, or DMSO just prior to use.

Assay procedure. To each well in a 96-well microtiter plate, 270 mL of assay buffer was added. Human α-thrombin (10 mL of 3 U mL^{-1}) was added, then 10 mL of inhibitor was added and mixed. The samples were incubated at room temperature for a defined period of time (3 min for initial IC_{50} determinations). The enzymatic reaction was initiated with 10 mL of 3 mM s-2238 substrate and continued at room temperature. The change in optical density was measured at 405 nm. A kinetic microplate reader (Molecular Devices Corporation u_{max}) was used to measure the change in optical density over time.

Results are reported in Table 1 as K_i values for different compounds. K_i was determined graphically from Dixon plots (plots of reciprocal enzyme velocity (1/v) versus inhibitor concentration at various enzyme substrate concentrations).

Procedure for determining the concentration required for doubling thrombin clotting time—clotting time assays. The following reagents were used in these assays: Owren's veronal buffer: 125 mM NaCl, 28.4 mM sodium barbital, pH 7.35; human citrated plasma obtained from human volunteers or citrated plasma obtained from dosed animals (prepared as described below); 25 NIH Units mL^{-1} human α-thrombin in thrombin buffer for use

with rat plasma; 10 NIH Units mL^{-1} human α-thrombin in thrombin buffer for use with human plasma.

Preparation of the citrated plasma. Human plasma: blood from human volunteers was drawn into vacutainer tubes containing one tenth final volume of 0.129 M (3.8%) buffered citrate (16 mg sodium citrate·$2H_2O$ and 2.1 mg citric acid per milliliter of H_2O). The blood was centrifuged at 3500 rpm (480 *g*) for 15 min at room temperature using a Sorvall RT 6000B centrifuge. The plasma was removed, pooled, and aliquoted into small tubes which were stored frozen for later use.

Dosing: test compound was prepared just prior to dosing. The drugs were routinely dissolved in water. Occasionally, other vehicles were used such as PEQ-200. Stock solutions were vortex-mixed and animals dosed po using a 3 mL syringe with an 18–19 gauge oral gavage needle or ip injection.

Blood drawing for rats: after the appropriate time period, the animals were ether-anesthetized, and blood was drawn by cardiac puncture using 333 mL of 3.8% sodium citrate per 3 mL blood. After all of the samples were obtained, the tubes were centrifuged at 1,500 rpm for 15 min as described for the human blood samples.

Clotting time measurement. Clotting times were determined by pipetting 0.1 mL of Owren's buffer (pre-warmed to 37 °C) and 0.1 mL of human or rat plasma into yellow sample cuvettes. For studies with human plasma, 10 U mL^{-1} human thrombin (10 mL) was placed in the reservoir assembly station of the MLA 700 (Medical Laboratory Automation, Electra 700 Reservoir Assembly). For rat studies, the human thrombin concentration was 25 U mL^{-1}. The cuvettes were vortexed and then placed on the MLA 700 sample wheel. The coagulation timer (MLA 700) automatically dispensed 0.1 mL human thrombin into the sample in each cuvette. Detection of the fibrin clot was determined optically by the MLA 700 instrument.

Studies were performed to determine the concentration of drug which caused a doubling of the clotting time (DCT) in human plasma. From standard curves of thrombin activity added to the sample versus the clotting time, the concentration of drug which caused a doubling of the thrombin clotting time corresponded to inhibition of approximately 1/2 of the added thrombin clotting activity.

Enzyme assays for the inhibition of trypsin. 3 mM Z-Val-Gly-Arg-*p*NA (Chromzyme TRY) dissolved in H_2O was used as the substrate for 6 μg mL^{-1} of purified bovine pancreatic trypsin dissolved in an assay buffer. The trypsin assay buffer comprised 2 mM $CaCl_2$, 50 mM Tris/Cl pH 8.0. Test compounds were dissolved in H_2O, methanol or DMSO immediately prior to use.

270 μL of assay buffer was added to each well in a 96-well microtiter plate followed by bovine trypsin (10 μL of a 6 μg mL^{-1} solution) and the test compound (10 μL). The mixture was incubated at room temperature for 3 min before initiating the enzymic reaction by introducing 10 μL of a 3 mM solution of the substrate, Z-Val-Gly-Arg-*p*NA. A kinetic microplate reader (Molecular Devices Corporation V_{max}) was used to measure the change in optical density at 405 nm over time.

Chemistry

General experimental procedures for selected compounds are given below. All anhydrous reactions were performed under an atmosphere of nitrogen using dry solvents from Aldrich Sure Seal bottles. Column chromatography was performed with silica gel 60 (E M Science, 230–400 mesh) using the mentioned solvent system as eluant. Thin-layer chromatography was conducted on Anatech GFLH or Whatman MK6F silica gel plates. Melting points were determined in an open capillary tube with a Thomas–Hoover melting point apparatus unless otherwise stated and are not corrected. Infrared spectra were recorded on a Perkin–Elmer 1800 Fourier transform spectrophotometer as thin films or KBr pellets. ^1H NMR spectra and ^{13}C NMR spectra were recorded on either Bruker AM-300 or Bruker AC-300 instruments and are expressed as parts per million (ppm or δ) from the mentioned solvent as the internal standard. Coupling constants are in hertz and signals are quoted as singlet (*s*), triplet (*t*), quartet (*q*), multiplet (*m*), and broad (*br*). Mass spectra were determined either on a Finnigan Model 4500 Quadrapole mass spectrometer by direct chemical ionization (DCI) with isobutane as the positive CI gas or by fast atom bombardment (FAB) on a Kratos MS-25 instrument. High resolution mass spectra (HRMS) were determined on a Kratos MS-50 mass spectrometer using the fast atom bombardment method with CsI in glycerol as the reference agent.

*Phenylmethyl [4-[[[5-(dimethylamino)-1-naphthalenyl]-sulfonyl]amino]-5-[4-ethyl-1-piperidinyl]-5-oxopentyl]-carbamate (**19d**).* To a cold (0 °C), nitrogen-blanketed mixture of 2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-5-[[(phenylmethoxy)carbonyl]amino]pentanoic acid[5] (48.90 g, 0.098 mol), triethylamine (25.0 mL, 0.18 mol) and 4-ethylpiperidine (20.0 g, 0.18 mol) in anhydrous dimethylformamide (160 mL) was added dropwise diphenylphosphoryl azide (21.1 mL, 0.098 mol). The mixture was stirred at 0 °C for 1 h and at ambient temperature for 2 h before it was partitioned between ether (150 mL) and water (80 mL). The ethereal layer was separated and the aqueous layer was extracted four additional times with ether (80 mL). The combined ethereal extracts were then washed with saturated sodium bicarbonate solution and brine prior to drying (Na_2SO_4) and solvent concentration. Two identical reactions were performed in tandem. Purification of the combined residues by HPLC on silica gel (elution with 50% ethyl acetate in hexane) furnished 53.09 g (45%) of the title compound as a yellow foam, mp 56–62 °C (dec.); ^1H NMR ($CDCl_3$) δ 8.47 (*d*, 1H), 8.30 (*t*, 1H), 8.16 (*d*, 1H), 7.58 (*m*, 1H), 7.56–7.36 (*m*, 6H), 7.15

(*d*, 1H), 6.17–5.96 (*dd*, 1H), 5.10 (*s*, 2H), 4.75–4.62 (*br m*, 1H), 4.10–3.91 (*br m*, 2H), 3.42–3.30 (*br m*, 1H), 3.16–2.92 (*br m*, 2H), 2.85–2.83 (*d*, 6H), 2.68–2.41 (*dt*, 1H), 2.24–1.97 (*m*, 1H), 1.57–0.76 (*m*, 3H), 0.16–0.08 (*m*, 1H); ^{13}C NMR ($CDCl_3$) ppm 168.50, 156.40, 151.62, 136.50, 135.00, 134.70, 130.56, 130.44, 129.80, 129.35, 129.18, 128.50, 128.41, 128.07, 128.00, 123.01, 122.93, 119.33, 115.58, 115.47, 66.60, 52.29, 52.19, 45.70, 45.43, 45.37, 45.13, 42.02, 42.21, 40.35, 40.18, 37.48, 37.34, 32.17, 31.92, 31.06, 30.79, 30.65, 29.67, 28.77, 28.58, 25.12, 11.08; IR (KBr, cm^{-1}) 3350, 2975, 2950, 1725, 1650, 1550, 1475, 1350, 1275, 1180, 1160; MS m/z (MH^+) 595. Anal. calcd for $C_{32}H_{42}N_4O_5S$: C, 64.62; H, 7.11; N, 9.41; found: C, 64.07; H, 7.29; N, 9.23.

*1-[5-Amino-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine dihydrochloride hydrate (**20d**).* Phenylmethyl [4-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-5-[4-ethyl-1-piperidinyl]-5-oxopentyl]carbamate (**19d**) (24.00 g, 0.040 mol) was added in one portion to a well-stirred, nitrogen-blanketed suspension of 10% palladium on carbon in a 5% formic acid/methanol mixture (350 mL). The reaction mixture was stirred at room temperature for 3 h before it was suction-filtered through Celite. The solvent was removed *in vacuo* and the residue was taken up in dichloromethane, washed with 1 N NaOH and brine prior to drying (Na_2SO_4) and solvent evaporation. Two identical reactions were performed in tandem. The free amine was obtained in 85% total yield (34.86 g) as a yellow foam and a small portion was quantitatively converted to the title compound with 20% HCl in ethanol; off-white solid, mp 161–165 °C (dec.); ^{1}H NMR (D_2O) δ 8.70–8.62 (*m*, 1H), 8.46–8.41 (*m*, 1H), 8.37–8.29 (*m*, 1H), 8.04–8.00 (*m*, 1H), 7.88–7.81 (*m*, 2H), 4.29–4.26 (*m*, 1H), 3.86–3.51 (*m*, 2H), 3.44 and 3.42 (2 *s*, 6H), 3.38–3.32 and 2.86–2.78 (2 *m*, 1H), 3.00–2.96 (*m*, 2H), 2.49 and 2.21 (2 *m*, 1H), 1.84–1.50 (*m*, 6H), 1.41–1.36 (*m*, 1H), 1.19–0.97 (*m*, 3H), 0.79–0.61 (*m*, 4H), 0.29–0.18 and 0.00–0.22 (2 *m*, 1H); ^{13}C NMR (D_2O) ppm 168.79, 168.71, 139.85–119.54 (19 lines, olefinic), 66.09, 52.48, 52.17, 46.94, 46.16, 45.93, 42.96, 42.71, 39.10, 36.59, 36.48, 31.91, 31.68, 31.06, 30.67, 29.51, 29.39, 28.62, 27.96, 23.31, 23.22, 14.29, 10.50; IR (KBr, cm^{-1}) 3573–3390, 2933, 1631, 1144, 796, 590; MS m/z (MH^+ – 2HCl) calcd for $C_{24}H_{37}N_4O_3S$ 461.2586; obsd 461.2584.

*1-[5-[(3-Amino-1,2,5-thiadiazol-4-yl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine S-oxide (**8d**).* A suspension of free amine **20d** (12.5 g, 0.085 mmol) in methanol (55 mL) was stirred at ambient temperature for 8 h. At this time, an additional 10.5 g of the thiadiazole-1-oxide was added. The suspension was stirred for a total of 48 h before it was suction-filtered and concentrated. Purification of the residue by flash chromatography on silica gel (elution with ethyl acetate followed by 10% methanol in dichloromethane) afforded 10.89 g (78% based on recovered starting material) of the title compound as a yellow foam, mp 158–168 °C (decomposes at 173 °C); ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 8.47 (*d*, *J* = 8.5 Hz, 1H), 8.30 (*d*, *J* = 8.6 Hz, 1H), 8.15–7.49 (*m*, 6H), 7.18 (*d*, *J* = 7.5 Hz, 1H), 4.11–3.88 (*m*, 2H), 3.52–3.48 (*m*, 1H), 3.34–3.26 (*m*, 3H), 2.84 (*s*, 6H), 2.77–1.88 (*m*, 2H), 1.69-1.34 (*m*, 6H), 1.17-0.98 (*m*, 3H), 0.83-0.76 (*m*, 4H), 0.24-0.12 (*m*, 1H); ^{13}C NMR ($CDCl_3$/DMSO-d_6) ppm 167.74, 167.64, 157.55, 157.32, 150.86, 135.42, 135.26, 129.51, 129.42, 128.92, 128.81, 128.21, 128.07, 127.37, 127.30, 122.67, 122.54, 119.02, 114.70, 114.57, 51.38, 51.26, 44.98, 44.78, 44.73, 44.55, 42.50, 42.36, 41.72, 41.20, 36.60, 31.59, 31.26, 30.57, 30.44, 29.76, 29.55, 28.14, 27.97, 23.76, 10.58; IR (KBr, cm^{-1}) 3375, 3237, 2937, 1614, 1583, 1149, 1064, 795, 629; MS m/z (MH^+) calcd for $C_{26}H_{38}N_7O_4S_2$ 576.2427, obsd 576.2441.

*1-[5-[(2-Amino-1,2-diiminoethyl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine trihydrochloride hydrate (**11d**).* Concentrated hydrochloric acid (6.1 mL) was added to a mixture of **8d** (5.44 g, 9.48 mmol) in methanol (87 mL) at room temperature. The reaction mixture was stirred for 3 h before additional hydrochloric acid (1 mL) was added in order to complete the reaction. Evaporation of the solvent after 7 h gave 6.05 g (100%) of the title compound as a pale-yellow solid, mp 175–179 °C (decomposes at 192 °C); ^{1}H NMR (D_2O) δ 8.77–8.69 (*m*, 1H), 8.52–8.46 (*m*, 1H), 8.38–8.31 (*m*, 1H), 8.15–8.11 (*m*, 1H), 7.95–7.86 (*m*, 2H), 4.34–4.25 (*m*, 1H), 3.76–3.60 (*m*, 2H), 3.53 (*s*, 6H), 3.42–3.35 (*m*, 1H), 2.91–2.27 (*m*, 1H), 1.94–1.54 (*m*, 5H), 1.43–1.39 (*m*, 1H), 1.21 and 0.99 (2 *m*, 4H), 0.81–0.71 (*m*, 3H), 0.30–0.25 and –0.08–(–0.15) (2 *m*, 1H); ^{13}C NMR (D_2O) ppm 171.98, 171.89, 159.38, 156.88, 142.07–123.03 (17 lines, olefinic), 60.83, 58.65, 55.70, 55.37, 50.32, 50.27, 49.42, 49.21, 46.44, 46.17, 45.96, 39.81, 39.69, 35.19, 34.92, 34.29, 33.91, 32.95, 32.81, 31.87, 31.21, 26.15, 26.06, 20.24, 13.76; IR (KBr, cm^{-1}) 3412, 2961, 1693, 1631, 1465, 1146, 797, 591; MS m/z (MH^+ – 3HCl) calcd for $C_{26}H_{40}N_7O_3S_1$ 530.2913; obsd 530.2902.

*1-[5-[[(Cyanoimino)(methylthio)methyl]amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine (**21**).* Dimethyl *N*-cyanodithioiminocarbonate (0.41 g, 2.18 mmol) was added in one portion to a mixture of **20d** (*n* = 3) hydrochloride (1.50 g, 2.81 mmol), 10% aqueous sodium hydroxide (2.2 mL, 6.05 mmol), and water (0.8 mL) in absolute ethanol (10 mL). The mixture was stirred at room temperature for 3 h before the ethanol was removed *in vacuo* and ethyl acetate was added. The organic phase was then separated, washed with brine, dried over sodium sulfate and evaporated. Purification of the residue by flash chromatography on silica gel (elution with 70% ethyl acetate in hexanes) provided 1.16 g (74%) of the title compound as a fluorescent-green foam, mp 73–83 °C; ^{1}H NMR ($CDCl_3$) δ 8.52 (*d*, *J* = 8.5 Hz, 1H), 8.29–8.16 (*m*, 1H), 7.61–7.55 (*m*, 1H), 7.51–7.46 (*m*, 1H), 7.18 (*d*, *J* = 7.6 Hz, 1H), 6.24–6.21 and 6.07–6.05 (2 *m*, 1H), 4.14–3.99 (*m*, 2H), 3.37–3.31 (*m*, 3H), 2.86 (*s*, 3H), 2.84 (*s*, 3H), 2.85–2.72 and 2.30–2.22 (2 *m*, 1H), 2.54 (*m*, 3H), 1.73–1.36 (series of *m*, 6H), 1.21–1.18 (*m*, 2H), 1.07–1.02 (*m*, 1H), 0.84–0.77 (*m*,

4H), 0.14 (*m*, 1H); ^{13}C NMR ($CDCl_3$) ppm 168.00, 151.74, 134.41, 130.76-128.52 (9 lines, olefinic), 123.01, 122.94, 118.96, 115.56, 115.43, 60.33, 52.17, 45.79, 45.23, 43.13, 42.90, 42.31, 37.38, 32.13, 31.94, 31.03, 30.40, 28.73, 28.54, 24.22, 24.12, 14.38, 14.15, 14.11, 11.07; IR (KBr, cm^{-1}) 3256, 2934, 2873, 2179, 1737, 1639, 1558, 1436, 1164, 1146, 627; MS m/z (MH^+) calcd for $C_{27}H_{39}N_6O_3S_2$ 559.2525; obsd 559.2515.

1-[5-[(5-Amino-1,2,4-oxadiazol-3-yl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine (5) and 1-[5-[(3-amino-1,2,4-oxadiazol-5-yl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine (6). To a solution of **21** (0.50 g, 0.90 mmol) and triethylamine (0.125 mL, 0.90 mmol) in absolute ethanol (5 mL) was added hydroxylamine hydrochloride (62.5 mg, 0.90 mmol). The mixture was stirred at room temperature for 1 h before additional triethylamine (0.50 mL, 3.60 mmol) and hydroxylamine hydrochloride (250 mg, 3.60 mmol) were added. After 3.5 days, the solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and water. The organic phase was then separated, concentrated down and subjected to gravity chromatography on silica gel (elution with 10% methanol in dichloromethane). There was isolated 134 mg (27%) of an inseparable mixture (8:2) of the title compounds as a fluorescent, greenish-yellow foam, mp 90–100 °C; ^1H NMR ($CDCl_3$) δ 8.48 (*d*, *J* = 8.4 Hz, 1H), 8.31 (*d*, *J* = 8.7 Hz, 1H), 8.18 (*d*, *J* = 6.2 Hz, 1H), 7.58–7.41 (*m*, 3H), 7.16 (*d*, *J* = 7.4 Hz, 1H), 6.21 and 5.68 (2 *br s*, 1H), 4.43 (*s*, 2H), 4.11–3.93 (*m*, 2H), 3.93–3.23 (2 *m*, 3H), 2.82 (*s*, 3H), 2.74–2.65 and 2.36 (2 *m*, 1H), 2.26–2.18 and 1.94–1.86 (2 *m*, 1H), 1.70–1.58 (*m*, 6H), 1.38–1.34 (*m*, 1H), 1.15–0.97 (2 *m*, 2H), 0.81–0.73 (*m*, 3H), 0.11 (*m*, 1H); ^{13}C NMR ($CDCl_3$) ppm 169.86, 168.75, 168.66, 168.29, 168.17, 151.59, 151.53, 135.51–115.42 (14 lines, olefinic), 53.45, 52.00, 51.77, 45.97, 45.43, 45.36, 42.71, 42.34, 42.14, 37.32, 32.23, 31.78, 31.11, 31.04, 30.79, 30.66, 28.69, 28.52, 25.23, 11.06; IR (KBr, cm^{-1}) 3335, 2937, 2873, 1631, 1589, 1577, 1454, 1315, 1163, 1146, 626, 575; MS m/z (MH^+) calcd for $C_{26}H_{37}N_7O_4S$ 544.2706; obsd 544.2701.

1-[5-[(3-Amino-1,2,5-thiadiazol-4-yl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]sulfonyl]amino]-1-oxopentyl]-4-ethylpiperidine S-dioxide (9). To a well-stirred solution of **20d** (1.00 g, 2.17 mmol) in dry methanol (10 mL) was added 3-methoxy-4-amino-1,2,5-thiadiazole-1-oxide (0.43 g, 2.60 mmol). The mixture was stirred for 2 h at ambient temperature before additional thiadiazole-1-oxide (0.43 g) was added. After 60 h, the mixture was concentrated down *in vacuo* and the residue was subjected to flash chromatography on silica gel (elution with 2% methanol in dichloromethane). There was isolated 0.40 g (31%) of the title compound as a pale yellow solid, mp 154–162 °C; ^1H NMR (CD_3SOCD_3) δ 8.93 (*br s*, 1H), 8.53 (*m*, 1H), 8.41 (*d*, *J* = 8.3 Hz, 1H), 8.29–8.26 (*m*, 1H), 8.15–8.08 (*m*, 2H), 7.95 (*m*, 1H), 7.58–7.51 (*m*, 2H), 4.12 (*br s*, 1H), 3.89–3.79 (*m*, 1H), 3.60 (*br m*, 1H), 3.22–3.15 (*m*, 2H), 2.79 (*s*, 6H), 2.72–2.68 and 2.48 (2 *m*, 1H), 2.23–2.15 and 1.91 (2 *m*, 1H), 1.48–1.34 (*m*, 6H), 1.12–1.10 (*m*, 2H), 0.98–0.93 (*m*, 1H), 0.78–0.70 (*m*, 4H), 0.26–0.22 and 0.08–0.05 (2 *m*, 1H); ^{13}C NMR (CD_3SOCD_3) ppm 168.06, 156.15, 155.89, 151.20, 136.27, 129.63-115.07 (12 lines, olefinic), 54.98, 51.67, 45.15, 45.11, 44.88, 43.27, 43.09, 41.85, 41.44, 36.84, 31.95, 31.64, 30.99, 30.82, 29.94, 29.65, 28.49, 28.27, 23.98, 23.90, 11.08, 11.03; IR (KBr, cm^{-1}) 3366, 2937, 1679, 1639, 1603, 1463, 1313, 1164, 1147, 793, 654, 627, 576; MS m/z (MH^+) calcd 592.2376; obsd 592.2369. Anal. calcd for $C_{26}H_{37}N_7O_5S_2 \cdot 0.5MeOH \cdot 0.1H_2O$: C, 52.22; H, 6.48; N, 16.09; H_2O, 0.30; found: C, 51.47; H, 6.18; N, 16.02.

N^{α}-[5-Dimethylamino-1-naphthalenesulfonyl]-N^{ω}-[benzyloxycarbonyl]-L-arginine lactam (17a). 5-Dimethylamino-1-naphthalenesulfonyl chloride (0.41 g, 1.53 mmol) was added in one portion to a stirred solution of N^{ω}-[benzyloxycarbonyl]-L-arginine lactam dihydrochloride hydrate (**16**) (0.50 g, 1.53 mmol) and 2 N NaOH (1.6 mL) in tetrahydrofuran (10 mL). The mixture was stirred at room temperature for 6 h before it was diluted with ethyl acetate and washed with saturated sodium bicarbonate solution and brine prior to drying and solvent concentration. Purification of the residue by gravity chromatography over silicia gel (gradient elution with 5% ethyl acetate in hexanes followed by 10% and finally 50% ethyl acetate in hexanes) afforded 0.26 g (33%) of the title compound as a yellow foam, mp 60–70 °C; ^1H NMR ($CDCl_3$) δ 9.23 (*br s*, 1.5H), 8.53 (*d*, *J* = 8.5 Hz, 1H), 8.27–8.20 (*m*, 2H), 7.61–7.47 (*m*, 2H), 7.36–7.24 (*m*, 8H), 7.19–7.17 (*d*, *J* = 7.6 Hz, 1H), 5.91 (*d*, *J* = 4.0 Hz, 1H), 5.08 (*s*, 2H), 4.78–4.70 (*m*, 1H), 3.97–3.90 (*m*, 1H), 3.28–3.18 (*m*, 1H), 2.86 (*s*, 6H), 2.38–2.30 (*m*, 1H), 1.77–1.72 (*m*, 2H); ^{13}C NMR ($CDCl_3$) ppm 174.28, 163.37, 159.48, 151.66, 136.33, 134.28, 130.80, 129.82, 129.41, 128.18, 129.08, 128.53, 128.38, 128.12, 122.99, 122.85, 118.57, 118.48, 115.32, 115.23, 67.08, 54.16, 53.95, 45.34, 45.20, 41.26, 41.12, 26.18, 26.01, 19.08; IR (KBr, cm^{-1}) 3373, 3275, 2944, 2871, 2833, 2788, 1697, 1613, 1507, 1478, 1456, 1375, 1320, 1263, 1180, 1163, 1138, 1094, 791, 629; MS m/z (MH^+) calcd 524.1968; obsd 524.1974. Anal. calcd for $C_{26}H_{29}N_5O_5S \cdot 0.1H_2O$: C, 59.44; H, 5.61; N, 13.33; H_2O, 0.34; found: C, 59.16; H, 5.97; N, 12.78; H_2O, 0.26.

N^{α}-[5-Dimethylamino-1-naphthalenesulfonyl]-L-arginine lactam dihydrochloride hydrate (3a). N^{α}-[5-Dimethylamino-1-naphthalenesulfonyl]-N^{ω}-[benzyloxycarbonyl]-L-arginine lactam (**17a**) (0.18 g, 0.34 mmol) was added in one portion to a well-stirred, nitrogen-blanketed suspension of 10% palladium on carbon in a 5% formic acid/methanol mixture (20 mL). The reaction mixture was stirred at room temperature for 5 h before it was suction-filtered through Celite. The residue was then taken up in dichloromethane, treated with 20% HCl in ethanol (10 mL) and evaporated down to dryness. There was isolated 163 mg (100%) of the title compound as a white foam, mp 128–178 °C (dec., sealed tube); ^1H NMR (D_2O) δ 8.80–8.76 (*m*, 1H), 8.50–8.40 (*m*, 2H),

8.10 (*d*, *J* = 7.8 Hz, 1H), 7.97–7.88 (*m*, 2H), 4.35–4.32, 4.03–3.93 and 3.75–3.70 (3 *m*, 1H), 3.51 and 3.50 (2 *s*, 6H), 3.09–2.96 (*m*, 1H), 1.99–1.89 (*m*, 1H), 1.82–1.40 (series of *m*, 3H); ^{13}C NMR (D_2O) ppm 168.01, 167.64, 151.35, 151.08, 133.51, 133.43, 133.37, 129.30, 125.66, 124.86, 123.18, 122.70, 122.62, 121.40, 120.85, 120.67, 120.53, 120.44, 120.15, 120.02, 113.99, 113.92, 49.95, 49.20, 46.95, 41.34, 41.06, 41.00, 34.61, 23.08, 20.69, 18.61, 14.15; IR (KBr, cm^{-1}) 3345, 3149, 1741, 1668, 1515, 1475, 1329, 1179, 1145, 1096, 1050, 795, 590; MS m/z (MH^+ – 2HCl) calcd for $C_{18}H_{24}N_5O_3S{\cdot}2HCl$ 390.1599; obsd 390.1581.

1-[3-[1-(Aminoiminomethyl)-4-piperidinyl]-2-[[[5-(dimethylamino)-1-napthalenyl]sulfonyl]amino]-1-oxopropyl]piperidine (2a). A solution of **12a** (1.55 g, 3.3 mmol) in methanol (30 mL) and 10% HCl/MeOH (3 mL) was hydrogenated in a Parr apparatus vessel using PtO_2 (0.2 g) as a catalyst for a period of 40 h. The solution was filtered through a pad of Celite and concentrated to give a yellow solid. Recrystallization from ethanol gave 1.45 g of pure piperidine hydrochloride **13a** which was used directly in the next step. A solution of **13a** (1.0 g, 2.1 mmol) in THF (20 mL) was reacted with excess *N*-CBz thioisocyanate at room temperature. The reaction was monitored by TLC. After 20 h, the reaction mixture was worked-up by adding water and extracting with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by SiO_2 chromatography using ethyl acetate:hexane (1:2) as eluant furnished **14a** (0.9 g) which was directly taken to the next step. To a stirred solution of **14a** (0.85g, 1.3 mmol) in THF (20 mL) was added one equivalent of 1 N NaOH and CH_3I. After 6 h, the mixture was quenched with water and subsequently extracted with ethyl acetate. Removal of the organic solvent and purification of the residue by SiO_2 chromatography gave 0.40 g of the desired product **15a**. Dry ammonia was then bubbled into a cold ethanolic solution of **15a** (0.40 g, 0.6 mmol) and the resulting mixture was stirred for 16 h at room temperature. Evaporation of the solvent gave the penultimate compound (0.36 g) as a foam. This was taken to the next step without any further purification. The CBz protected compound was taken up in CH_3OH (20 mL) and 100 mg of 10% Pd/C was added. The mixture was shaken in a Parr hydrogenator at 50 psi for a period of 8 h. The solution was filtered and evaporated to give the title compound (0.12 g) as a foam, mp 145–155 °C (dec.); ^1H NMR ($CDCl_3$) δ 8.50 (*d*, 1H), 8.27 (*d*, 1H), 8.14 (*d*, 1H), 7.69–7.14 (*m*, 3H), 4.19–3.94 (m, 5H), 3.15–3.82 (*m*, 5H), 2.97 (*s*, 6H), 2.01–0.80 (*m*, 13H); IR (film, cm^{-1}) 3240, 2960, 2880, 2400, 2280, 1725, 1650, 1450, 1180 1160, 920, 800; MS m/z (MH^+) calcd for $C_{26}H_{38}N_6O_3S$ 514.2726; obsd 514.2733.

1-[5-(4-Amino-1,2,5-thiadiazol-3-yl)amino]-2-[[[5-(dimethylamino)-1-naphthalenyl]amino]-1-oxopentyl]-4-ethylpiperidine (7). To a solution of bis-amidine **11d** (1.6 g, 2.5 mmol) in CH_2Cl_2 (10 mL) and triethylamine (7.5 mmol) was added *N,N'*-thiobisphthalimide. The reaction mixture was stirred for 3 h and was worked-up by washing with 1 N NaOH and drying over Na_2SO_4. After concentration of the organic layer, the residue was purified by SiO_2 chromatography using CH_2Cl_2:MeOH:NH_4OH (90:10:1) to yield the title compound (1.3 g) as an off-white powder, mp 147–148 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.55 (*d*, 1H), 8.33 (*dd*, 1H), 8.17 (*d*, 1H), 7.57 (*m*, 1H), 7.50 (*m*, 1H), 7.21 (*m*, 1H), 6.34–6.20 (*dd*, 1H), 4.88 (*br s*, 2H), 4.29–4.22 (*dd*, 1H), 4.21–4.18 (*dd*, 1H), 3.39–3.30 (*m*, 3H), 2.87 (2 *s*, 6H), 2.71–2.68 (*t*, 1H), 2.49 and 1.98 (2 *t*, 1H), 1.76–1.69 (*m*, 3H), 1.59–1.55 (*m*, 3H), 1.49–1.47 (*d*, 1H), 1.39–1.37 (*m*, 2H), 1.18–1.13 (*m*, 4H), 0.81–0.77 (*m*, 4H), 0.28–0.18 (*m*, 1H); IR (film, cm^{-1}) 3346, 3230, 2934, 1632, 1568, 1537, 1476, 1454, 1314, 1144, 1062, 912, 792, 732; MS m/z (MH^+) 560. Anal. calcd for $C_{26}H_{37}N_7O_3S_2$: C, 55.72; H, 6.54; N, 17.37; found: C, 55.79 H, 6.66; N, 17.52.

References and Notes

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