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Novel thrombin inhibitors with azaphenylalanine scaffold

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Dedicated to Professor Richard Neidlein, Heidelberg, on the occasion of his 70th anniversary

In this paper the synthesis and antithrombotic activity of a series of novel thrombin inhibitors with azaphenylalanine scaffold are described. By systematic structural modifications for this series we have identified optimal groups for achieving nanomolar potency, that led to potent inhibitors of thrombin with Ki values up to 11 nM.

1. Introduction

Thrombin plays a major role in thrombosis, which is one of the leading causes of cardiovascular disease and morbidity in developed societies [1]. Not surprisingly, the development of low molecular weight thrombin inhibitors has become a subject of extensive research [1–7].

In the course of our ongoing research programme directed towards the design and synthesis of thrombin inhibitors, we have incorporated an azapeptide scaffold into the central part of the argatroban-like thrombin inhibitor structure [6]. The α-carbon of the original peptidomimetic structure was replaced with nitrogen and the stereogenic center of the central amino acid was omitted, with the result that the overall conformation was changed. Incorporation of the azapeptide scaffold can favorably alter the inhibitor properties with regard to stability, enzymatic degradation, absorption and transport [8, 9].

2. Investigations, results and discussion

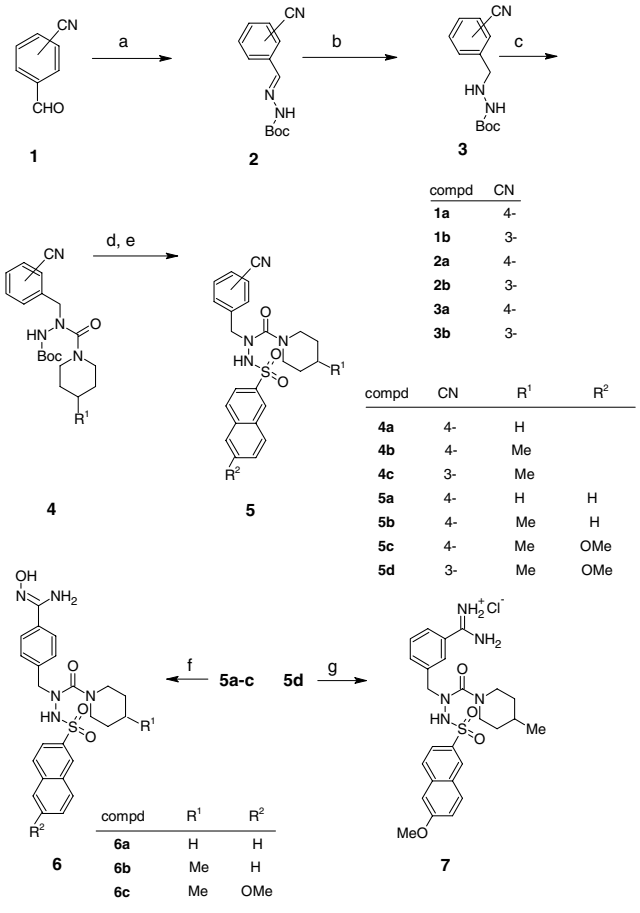
In this paper, we outline the transformation of the parent compound **6a** with modest thrombin inhibitory potency into **7**, a highly potent thrombin inhibitor.

The target compounds listed in the Table were synthesized as outlined in the Scheme.

Commercially available 4-cyanobenzaldehyde and 3-cyanobenzaldehyde (**1**) were transformed into the corresponding Boc-protected hydrazones **2** using *tert*-butylcarbazate followed by catalytic hydrogenation on Pd/carbon to give the intermediates **3**. Coupling of these Boc-protected hydrazines **3** with cyclic amines gave the required Boc-protected carbazamides **4** in a one-pot synthesis using commercially available triphosgene. Deprotecting the Boc group in compounds **4** by treatment with gaseous HCl in AcOH and subsequent reaction with aryl sulfonyl chloride led to compounds **5**. Methoxy-substituted naphthalenesulfonyl chloride for compounds **5c, d** was prepared from 6-hydroxy-2-naphthalenesulfonic acid sodium salt and thionyl chloride according to literature procedures [10, 11]. Finally, hydroxylamine was used to convert the nitriles **5** to the target benzamidoximes **6a–c**, and the cyano compound was treated with ethanolic HCl and reacted with ammonium acetate to give the corresponding amidine **7**.

Methylation of the piperidine ring resulted in increased hydrophobicity at the P2 part of the molecule, resulting in a 2-fold increase in the potency of **6b** compared to **6a**. Encouraged by the sub-micromolar activity observed with this compound, our attention moved to the variation of its sulfonamide aryl moiety. Replacement of the naphthyl group with the 6-methoxy-2-naphthyl group led to compound **6c** which also demonstrated enhancement of potency as compared to **6b**.

Scheme



a) Boc-$NHNH_2$, EtOH, reflux; (b) H_2, Pd/C, MeOH; (c) 1. $(Cl_3CO)_2CO$, CH_2Cl_2, 2. cyclic amine, DIEA; (d) HCl (g), AcOH; (e) aryl sulfonyl chloride, CH_2Cl_2, Et_3N; (f) NH_2OH, EtOH, reflux; (g) HCl/EtOH, NH_4OAc

Table 1: Inhibition of thrombin by novel thrombin inhibitors with an azaphenylalanine scaffold and by the established thrombin inhibitor, argatroban

Compd.	Ki (μM)	TT* (μM)	aPTT* (μM)	PT* (μM)
6a	1.938	2126	3045	2702
6b	0.768	1372	2613	1103
6c	0.378	712	369	52
7	0.011	2.0	2.1	2.7
Argatroban	0.008	0.2	2.6	2.8

* Concentration of inhibitor doubling the respective clotting time

Replacing the amidoximo moiety with an amidino moiety and concomitant change of its position on the aromatic ring in the P1 part of the molecule resulted in **7** with Ki = 0.011 μM. Some of the new thrombin inhibitors with an azaphenylalanine scaffold exert high anticoagulant activity having nanomolar Ki values (Table).

In summary, a series of structural modifications of the parent compound **6a** resulted in 485-fold enhancement of thrombin inhibitory activity and the anticoagulant potency of compound **7** is higher, or at least in the same range, as that of argatroban.

3. Experimental

3.1. Enzyme assay

Inhibition of thrombin was measured spectrophotometrically in microtiter plates at room temperature, using chromogenic substrates. Inhibitors were dissolved in dimethylsulfoxide (DMSO) and diluted with buffer or water. For compounds **6a–c** human thrombin was tested at 1 nM f.c. with the substrate S2366 (Km 108 μM) at 200 μM f.c. using HNPT buffer (Hepes 100 mM, Na Cl 140 mM, PEG 6000 0.1% Tween 80 0.02%, pH 7.8). For compound **7** human thrombin was tested at 0.5 NIHU/mL f.c. with substrate S2238 (Km 2.6 μM) at 20 μM f.c. using HBSA buffer (Hepes 10 mM, NaCl 150 mM, bovine serum albumin 0.1% w/v, pH 7.5). Values of the Ki were calculated according to Cheng and Prussof 12 based on IC50 or from relation between reaction velocity equations in the absence and presence of inhibitor using the relevant Km.

3.2. Effect on plasma coagulation

The *in vitro* anticoagulant effect of novel thrombin inhibitors was measured by thrombin time (TT), activated partial thromboplastine time (aPTT), and prothrombin time (PT) assays. Briefly, inhibitors were added to normal pooled human plasma over a range of concentrations and clotting was recorded in an automated coagulometer (Fibrintimer, Dade/Behring). The concentration of inhibitor that doubled the clotting time was determined for each assay.

3.3. Chemistry

Melting points were determined on a Kofler hot stage microscope (Reichter, Co.) and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance DPX_{300} spectrometer or a Varian INOVA 600 MHz spectrometer. The proton chemical shifts of compounds were assigned following standard procedures using homonuclear DQF-COSY, TOCSY, and NOESY experiments and are reported in δ [ppm] relative to tetramethylsilane. The symbol "Pip" in NMR assignments refers to piperidine, "Ar" to phenyl and "Nph" to naphthalene ring. The IR spectra were recorded on a Perkin Elmer FTIR 1600 spectrophotometer. Mass spectroscopy was performed on a Varian-MAT 311A mass spectrometer. All solvents and reagents used in the syntheses were of commercial synthetic grade and when required were further purified and dried by standard methods. All reactions with air- or moisture-sensitive reactants were carried out under argon atmosphere. DIEA stands for *N*,*N*-diisopropylethylamine.

3.3.1. General procedure for the synthesis of compounds **2a** *and* **2b**

4-Cyanobenzaldehyde or 3-cyanobenzaldehyde (40.0 mmol, **1**) suspended in EtOH (100 ml) was added to a stirred solution of tert-butylcarbazate (40.0 mmol) in EtOH. The mixture was heated at reflux temperature and after 4 h the EtOH was partially evaporated in vacuo. Water (100 ml) was added and the precipitated product was collected by filtration and washed with diethylether to give the appropriate Boc-protected hydrazone **2** as a white solid.

3.3.2. Tert-butyl-2-(4-cyanobenzylidene)hydrazinecarboxylate (**2a**)

Yield: 95%; m.p. 158–160 °C (lit.154–158 °C) 13; IR (KBr) 3297, 2996, 2227, 1701, 1531, 1459, 1374, 1258, 1149, 1058, 946, 833, 772, 610 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 1.56 (s, 9 H, Boc-H), 7.65 (d, J = 8.3 Hz, 2 H, Ar-$H^{2,3}$), 7.77 (d, J = 8.3 Hz, 2 H, Ar-$H^{5,6}$), 7.93 (s, 1 H, CH), 8.13 (s, 1 H, NH); FAB MS: $MH^+ = 246$.
$C_{13}H_{15}N_3O_2$ (245.3)

3.3.3. Tert-butyl-2-(3-cyanobenzylidene)hydrazinecarboxylate (**2b**)

Yield: 77%; m.p. 115–158 °C; IR (KBr) 3297, 2977, 2232, 1703, 1530, 1477, 1376, 1248, 1160, 1061, 941, 865, 796, 684 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 1.55 (s, 9 H, Boc-H), 7.55 (m, 2 H, Ar-H), 7.93 (m, 3 H, Ar-H and CH), 8.24 (s, 1 H, NH); FAB MS: $MH^+ = 246$.
$C_{13}H_{15}N_3O_2$ (245.3)

3.3.4. General procedure for the synthesis of compounds **3a** *and* **3b**

The corresponding hydrazone **2** (60.0 mmol) was dissolved in MeOH (250 ml) and 10% Pd/C (10 w/w%) was added. The mixture was hydrogenated for 6 h. The catalyst was filtered off and the filtrate was evaporated in vacuo to yield oily intermediate **3** which crystallized overnight.

3.3.5. Tert-butyl 2-(4-cyanobenzyl)hydrazinecarboxylate (**3a**)

Yield: 97%; m.p. 58–64 °C (lit. 66–69 °C) [13]; IR (KBr) 3366, 3236, 2984, 2932, 2225, 1685, 1484, 1368, 1284, 1166, 1023, 818 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 1.45 (s, 9 H, Boc-H), 4.05 (d, J = 4,5 Hz, 2 H, CH_2), 4.31 (br s, 1 H, NH), 6.18 (br s, 1 H, NH), 7.46 (d, J = 8,3 Hz, 2 H, Ar-$H^{2,6}$), 7.61 (d, J = 8,3 Hz, 2 H, Ar-$H^{3,5}$); FAB MS: $MH^+ = 248$.
$C_{13}H_{17}N_3O_2$ (247.3)

*3.3.6. Tert-butyl 2-(3-cyanobenzyl)hydrazinecarboxylate (***3b**)

Yield: 95%; m.p. 75–80 °C; IR (KBr) 3368, 2980, 2226, 1679, 1482, 1366, 1286, 1166, 793 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 1.47 (s, 9 H, Boc-H), 4.04 (br s, 2 H, CH_2), 4.28 (br s, 1 H, NH), 6.09 (br s, 1 H, NH), 7.12–7.41(m, 4 H, Ar-H); FAB MS: $MH^+ = 248$.
$C_{13}H_{17}N_3O_2$ (247.3)

3.3.7. General procedure for the synthesis of compounds **4a–c**

Triphosgene (23.5 mmol) was dissolved in CH_2Cl_2 (40 ml) and a mixture of Boc-protected hydrazine **3** (47.0 mmol) and DIEA (70.6 mmol) in CH_2Cl_2 (80 ml) was slowly added to the stirred solution of triphosgene over a period of 30 min. After a further 5 min of stirring, a solution of cyclic amine (47.0 mmol) and DIEA (70.6 mmol) in CH_2Cl_2 (80 ml) was added in one lot. The reaction mixture was stirred for 30 min, evaporated to dryness, diluted with EtOAc (100 ml), washed with 10% aqueous citric acid (50 ml), 10% aqueous $NaHCO_3$ (50 ml) and saline solution (50 ml). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to give oily Boc-protected carbazamide **4**. The product was crystallized from diethylether as white crystals.

*3.3.8. Tert-butyl 2-(4-cyanobenzyl)-2-(1-piperidinylcarbonyl) hydrazinecarboxylate (***4a**)

Yield: 42%; m.p. 50–55 °C; IR (KBr) 3294, 2942, 2229, 1730, 1638, 1519 cm^{-1}; 300.15 MHz ^{1}H NMR (DMSO-d_6): 1.28–1.60 (m, 6 H, Pip-$H^{3,4,5}$), 1.37 (s, 9 H, Boc), 2.97–3.20 (m, 4 H, Pip-$H^{2,6}$), 4.45 (br s, 2 H, CH_2), 7.50 (d, J = 8,3 Hz, 2 H, Ar-$H^{2,6}$), 7.78 (d, J = 8,3 Hz, 2 H, Ar-$H^{3,5}$), 9.27 (s, 1 H, NH); FAB MS: $MH^+ = 359$.
$C_{19}H_{26}N_4O_3$ (358.4)

*3.3.9. Tert-butyl 2-(4-cyanobenzyl)-2-[(4-methyl-1 piperidinyl)carbonyl] hydrazinecarboxylate (***4b**)

Yield: 81%; m.p. 55–58 °C; IR (KBr) 3284, 2977, 2228, 1728, 1636 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 0.96 (d, J = 6,4 Hz, 3 H, CH_3), 0.90–1.05 (m, 2H, Pip-$H^{3,5}$), 1.45 (s, 9 H, Boc), 1.45–1.60 (m, 4 H, Pip-$H^{3',5'}$), 2.60–2.75 (m, 2 H, Pip-$H^{2,6}$), 3.70–3.85(m, 2 H, Pip-$H^{2',6'}$), 4.52 (br s, 2 H, CH_2), 7.48 (d, J = 8,3 Hz, 2 H, Ar-$H^{2,6}$), 7.61 (d, J = 8,3 Hz, 2 H, Ar-$H^{3,5}$), 9.32 (s, 1 H, NH); FAB MS: $MH^+ = 373$.
$C_{20}H_{28}N_4O_3$ (372.5)

3.3.10. Tert-butyl 2-(3-cyanobenzyl)-2-[(4-methyl-1-piperidinyl) carbonyl] hydrazinecarboxylate (**4c**)

Yield: 31%; m.p. 147–150 °C; IR (KBr) 3275, 2923, 2229, 1722, 1632 cm^{-1}; 300.15 MHz ^{1}H NMR ($CDCl_3$): 0.88 (d, J = 6,0 Hz, 3 H, CH_3), 0.94–1.05 (m, 2 H, Pip-$H^{3,5}$), 1.36 (s, 9 H, Boc), 1.37–1.60 (m, 4 H, Pip-$H^{3',5'}$), 2.64–2.80 (m, 2 H, Pip-$H^{2,6}$), 3.72–3.85 (m, 2 H, Pip-$H^{2',6'}$), 4.43 (br s, 2 H, CH_2), 7.41–7.57 (m, 1 H, Ar-H), 7.59–7.78 (m, 3 H, Ar-H), 9.28 (s, 1 H, NH); FAB MS: $MH^+ = 373$.
$C_{20}H_{28}N_4O_3$ (372.5)

3.3.11. General procedure for the synthesis of compounds **5a–d**

HCl gas was bubbled for 45 min through a solution of Boc-protected carbazamide **4** (3.9 mmol) in AcOH (15 ml) at room temperature. AcOH was evaporated in vacuo to yield oily intermediat. To a solution of this compound (2.3 mmol) in CH_2Cl_2 (50 ml) cooled to 0 °C were added DIEA (7.1 mmol) and aryl sulfonyl chloride (2.3 mmol). The mixture was stirred overnight at RT and concentrated in vacuo. The residue was dissolved in EtOAc (100 ml) and washed with H_2O (50 ml), 1M HCl (50 ml) and brine (50 ml). The organic phase was dried over Na_2SO_4. After evaporation of the solvent under reduced pressure, the product **5** was crystallized from diethylether as white crystals.

3.3.12. N′-(4-Cyanobenzyl)-N-(1-piperidinylcarbonyl)-2-naphthalenesulfonohydrazide (**5a**)

Yield: 33%; m.p. 155–157 °C; IR (KBr) 2940, 2233, 1801, 1630 cm^{-1}; 300.15 MHz ^{1}H NMR (DMSO-d_6):1.04–1.45(m, 8H, Pip-H^{3,4,5}), 2.90–3.20 (m, 4 H, Pip-H^{2,6}), 4.29 (br d, 2 H, CH_2), 7.16(d, 2 H, J = 8.6 Hz, Ar-H^{2,6}), 7.65–7.77 (m, 5 H, Ar-H^{3,5} and Nph-H^{3,6,7}), 8.05–8.17 (m, 3 H, Nph-H^{4,5,8}), 8.43 (s, 1 H, Nph-H^1), 9.59 (s, 1 H, NH); FAB MS: $MH^+ = 449$.
$C_{24}H_{24}N_4O_3S$ (448.5)

3.3.13. N′-(4-Cyanobenzyl)-N′-[(4-methyl-1-piperidinyl)carbonyl]-2-naphthalenesulfonohydrazide (**5b**)

Yield: 22%; m.p. 148–151 °C; IR (KBr) 3191, 3054, 2913, 2224, 1686 cm^{-1}; 300.15 MHz ^{1}H NMR (DMSO-d_6): 0.10–0.35 (m, 2 H, Pip-H$^{3,3',5,5'}$), 0.47 (d, 3 H, J = 6.0 Hz, CH_3), 1.22–1.26 (m, 3 H, Pip-H$^{3,3',4,4',5,5'}$), 2.30–2.45 (m, 1 H, Pip-H$^{2,2'}$), 2.65–2.80 (m, 1 H, Pip-H$^{6,6'}$), 3.45–3.65 (m, 2 H, Pip-H$^{2,2',6,6'}$), 4,38 (br d, 2 H, CH_2), 7.34 (d, 2 H, J = 8.3, Ar-H^{2,6}), 7.64–7.80 (m, 5 H, Ar-H^{3,5} and Nph-H^{3,6,7}), 8.02–8.18 (m, 3 H, Nph-H^{4,5,8}), 8.42 (s, 1 H, Nph-H^1), 9.55 (s, 1 H, NH) (Two sets of signals). FAB MS: $MH^+ = 463$.
$C_{25}H_{26}N_4O_3S$ (462.6)

3.3.14. N′-(4-Cyanobenzyl)-6-methoxy-N′-[(4-methyl-1-piperidinyl)carbonyl]-2-naphthalenesulfonohydrazide (**5c**)

Yield: 34%; m.p. 154–156 °C; IR (KBr) 3175, 2928, 2225, 1654 cm^{-1}; 300.15 MHz ^{1}H NMR (DMSO-d_6): 0.10–0.47 (m, 2 H, Pip-H), 0.53 (d, 3H, J = 6.0 Hz, CH_3), 1.15–1.32 (m, 3 H, Pip-H), 2.30–2.45 (m, 1 H, Pip-H), 2.55–2.75 (m, 1 H, Pip-H), 3.42–3.65 (m, 2 H, Pip-H), 3.92 (s, 3 H, CH_3) 4,35 (br d, 2 H, CH_2), 7.28–7.39 (m, 3 H, Ar-H^{2,6} and Nph-H), 7.45 (d, 1 H, J = 2.26 Hz, Nph-H), 7.65–7.77 (m, 3 H, Ar-H^{3,5} and Nph-H), 7.94 (d, 1 H, J = 8.66 Hz, Nph-H), 8.05 (d, 1 H, J = 8.66 Hz, Nph-H), 8.34 (d, 1 H, J = 1.88, Nph-H), 9.42 (s, 1 H, NH); FAB MS: $MH^+ = 493$.
$C_{26}H_{28}N_4O_4S$ (492.6)

3.3.15. N′-(3-Cyanobenzyl)-6-methoxy-N′-[(4-methyl-1-piperidinyl)carbonyl]-2-naphthalenesulfonohydrazide (**5d**)

Yield: 26%; m.p. 169–173 °C; IR (KBr) 3168, 2932, 2232, 1671 cm^{-1}; 300.15 MHz ^{1}H NMR (DMSO-d_6): 0.15–0.40 (m, 2 H, Pip-H), 0.53 (d, 3H, J = 6.0 Hz, CH_3), 1.25–1.39 (m, 3 H, Pip-H), 2.25–2.38 (m, 1 H, Pip-H), 2.55–2.74 (m, 1 H, Pip-H), 3.45–3.65 (m, 2 H, Pip-H), 3.84 (s, 3 H, CH_3), 4.32 (br d, 2 H, CH_2), 7.28–7.33 (m, 1 H, Nph-H), 7.44–7.54 (m, 3H, Ar-H and Nph-H), 7.60 (d, 1 H, J = 1.88 Hz, Ar-H), 7.60–7.76 (m, 2H, Ar-H and Nph-H), 7.94 (d, 1 H, J = 8.66 Hz, Nph-H), 8.05 (d, 1 H, J = 8.66 Hz, Nph-H), 8.34 (d, 1 H, J = 1.88 Hz, Nph-H^1), 9.46 (s, 1 H, NH); FAB MS: $MH^+ = 493$.
$C_{26}H_{28}N_4O_4S$ (492.6)

3.3.16. General procedure for the synthesis of compounds **6a–c**

NH_2OH (16.4 mmol) was added to the solution of nitrile **5** (8.2 mmol) in EtOH (100 ml). The mixture was heated at reflux temperature overnight and evaporated under reduced pressure to yield corresponding benzamidoxime **6** as a white foam. Product was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 50 : 1).

3.3.17. N′-Hydroxy-4-{[2-(2-naphthylsulfonyl)-1-(1-piperidinylcarbonyl) hydrazino] methyl}benzenecarboximidamide (**6a**)

Yield: 38%; m.p. 194–197 °C; IR (KBr) 3383, 1660, 1427, 1335 cm^{-1}; 600 MHz ^{1}H NMR (DMSO-d_6): 1.03 (br s, 4 H, Pip-H^{3,5}), 1.24 (m, 2 H, J = 5.75 Hz, Pip-H^4), 2.93 (br s, 2 H, Pip-H^{2,6}), 3.06 (br s, 2 H, Pip-H$^{2',6'}$), 4.18 (br s, 1 H, $CH_2$1), 4.38 (br s, 1 H, $CH_2$2), 5.78 (s, 2 H, NH_2), 7.16 (d, 2 H, J = 8.2 Hz, Ar-H^{2,6}), 7.58 (d, 2 H, J = 8.2 Hz, Ar-H^{3,5}), 7.68 (dd, 1 H, J = 8.0, Nph-H^7), 7.71 (dd, 1 H, J = 8.0 Hz, Nph-H^6), 7.77 (d, 1 H, J = 8.6 Hz, Nph-H^3), 8.04 (d, 1 H, J = 8.0 Hz, Nph-H^5), 8.09 (d, 1 H, J = 8.0 Hz, Nph-H^4), 8.16 (d, 1 H, J = 8.0 Hz, Nph-H^8), 8.46 (s, 1 H, Nph-H^1), 9.41 (s, 1 H, NH), 9.61 (s, 1 H, OH). FAB MS: $MH^+ = 482$.
$C_{24}H_{27}N_5O_4S$ (481.6)

3.3.18. N′-Hydroxy-4-{[1-[(4-methyl-1-piperidinyl)carbonyl]-2-(2-naphthylsulfonyl)hydrazino]methyl}benzenecarboximidamide (**6b**)

Yield: 56%; m.p. 195–197 °C; IR (KBr) 3414, 2947, 1654 cm^{-1}; 600 MHz ^{1}H NMR (DMSO-d_6): 0.06 (br s, 1 H, Pip-H^5), 0.25 (br s, 1 H, Pip-H^3), 0.43 (d, 3 H, J = 6.0 Hz, CH_3), 1.20 (br s, 3 H, Pip-H$^{3',4',5'}$), 2.35 (br s, 1 H, Pip-H$^{2'}$), 2.65 (br s, 1 H, Pip-H$^{6'}$), 3.50 (br s, 1 H, Pip-H^6), 3.55 (br s, 1 H, Pip-H^2), 4.22 (br s, 1 H, $CH_2$2), 4.39 (br s, 1 H, $CH_2$1), 5.78 (s, 2 H, NH_2), 7.16 and 7.23 (d, 1 H, J = 8.2 Hz, Ar-H^{2,6}), 7.59 and 7.79 (d, 1 H, J = 8.2 Hz, Ar-H^{3,5}), 7.66 (dd, 1 H, J = 8.0, Nph-H^7), 7.71 (dd, 1 H, J = 8.0, Nph-H^6), 7.77 (d, 1 H, J = 8.0, Nph-H^3), 8.03 (d, 1 H, J = 8.0 Hz, Nph-H^5), 8.07 (d, 1 H, J = 8.0 Hz, Nph-H^4), 8.14 (d, 1 H, J = 8.0 Hz, Nph-H^8), 8.45 (s, 1 H, Nph-H^1), 9.39 and 9.46 (br s, 1 H, NH), 9.62 (s, 1 H, OH); FAB MS: $MH^+ = 496$.
$C_{25}H_{29}N_5O_4S$ (495.6)

3.3.19. N′-Hydroxy-4-({2-[(6-methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidinyl)carbonyl]hydrazino}methyl)benzenecarboximidamide (**6c**)

Yield: 62%; m.p. 78–80 °C; IR (KBr) 3375, 2925, 1645 cm^{-1}; 600 MHz ^{1}H NMR (DMSO-d_6): 0.24 (br d, 3 H, Pip-H), 0.43 (d, 3 H, J = 6.0 Hz, CH_3), 1.25 (br d, 3 H, Pip-H), 2.38 (br s, 1 H, Pip-H), 2.64 (br s, 1 H, Pip-H), 3.55 (br s, 2 H, Pip-H), 3.91 (s, 3 H, OCH_3), 4.30 (br d, 2 H, CH_2), 5.77 (s, 2 H, NH_2), 7.15 (d, 2 H, J = 8.3 Hz, Ar-H^{2,6}), 7.59 (d, 2 H, J = 8.3 Hz, Ar-H^{3,5}), 7.31 (dd, 1 H, J = 8.0, Nph-H), 7.41 (d, 1 H, J = 2.26, Nph-H), 7.71 (dd, 1 H, J = 8.3 Hz, Nph-H), 7.94 (d, 1 H, J = 8.0 Hz, Nph-H), 8.06 (d, 1 H, J = 8.0 Hz, Nph-H), 8.37 (d, 1 H, J = 8.0 Hz, Nph-H), 9.21 (br s, 1 H, NH), 9.60 (s, 1 H, OH); FAB MS: $MH^+ = 526$.
$C_{26}H_{28}N_4O_4S$ (525.6)

3.3.20. 3-({2-[(6-Methoxy-2-naphthyl)sulfonyl]-1-[(4-methyl-1-piperidinyl) carbonyl] hydrazino}methyl)benzenecarboximidamide hydrochloride (**7**)

Anhydrous hydrogen was bubbled into an ice-cooled suspension of **5d** (0.5 mmol) in EtOH (10 ml) for 30 min. The mixture was stirred for 6 h at room temperature and concentrated in vacuo. The residual solid was rinsed with isopropyl ether, dried under reduced pressure, and dissolved in 10 ml of EtOH. To this solution was added ammonium acetate (1.3 mmol), and the mixture was stirred for 20 h. The resulting precipitate was collected by filtration and treated with ethanolic HCl to afford 75 mg (30%) of corresponding amidine **7** as a white solid.
M.p. 151–154 °C; IR (KBr) 3054, 1677, 1624 cm^{-1}; 300 MHz ^{1}H NMR ($CDCl_3$): 0.28 (br d, 3H, Pip-H), 0.54 (d, 3H, J = 6.0 Hz, CH_3), 1.27 (br d, 3H, Pip-H), 2.45 (br s, 1 H, Pip-H), 2.64 (br s, 1 H, Pip-H), 3.66 (br s, 2 H, Pip-H), 3.95 (s, 3 H, OCH_3), 4.32 (m, 2 H, CH_2), 7.16–7.28 (m, 2 H, Nph-H), 7.36–7.40 (m, 2 H, Ar-H^{2,6}), 7.73–7.87 (m, 4 H, Ar-H^5 and Nph-H), 7.95 (m, 1 H, Ar-H^4), 8.78 (br s, 2 H, NH_2), 8.16 (br s, 2 H, NH_2); FAB MS: $MH^+ = 510$.
$C_{26}H_{31}ClN_5O_3S$ (545.1)

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