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Short Communication

Discovery of pyrimidyl-5-hydroxamic acids as new potent histone deacetylase inhibitors

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Abstract

A series of pyrimidyl-5-hydroxamic acids was prepared for evaluation as inhibitors of histone deacetylase (HDAC). Amino-2-pyrimidinyl can be used as a linker to provide HDAC inhibitors of good enzymatic potency.

Keywords: Histone deacetylase; Hydroxamic acid; Anticancer agents

1. Introduction

The past years have seen a growing interest in histone deacetylase (HDAC), enzymes which play a fundamental role in the regulation of gene expression [1].

By modifying the acetylation status of ϵ-amino groups of specific lysine residues on histones proteins complexed with DNA in the nucleosomes, HDACs regulate the transcriptional process. Inhibitors of HDAC activity from various structural families induce histone hyperacetylation, reactivate suppressed genes and consequently, inhibit the cell cycle, activate differentiation programs or induce apoptosis. Several HDAC inhibitors have shown inhibition of both cell proliferation and tumor growth in vivo, and few are currently undergoing phase I or phase II clinical evaluation [2–10]. HDAC inhibitors cause their biological effects through a large number of different mechanisms. Microarray experiments show that in response to HDAC inhibitors about 2% of all genes is induced [11]. One more general contributor to the anti-proliferative effects of HDAC inhibitors, however, appears to be the induction of the cyclin dependent kinase inhibitor $p21^{waf1,cip1}$. The crucial role of $p21^{waf1,cip1}$ has been demonstrated by studies showing a sixfold increase in resistance to the HDAC inhibitor trichostatin A (TSA) in $p21^{waf1,cip1}$ deficient cells as compared with the parental HCT-116 cells [12,13]. In addition, HDACs also regulate cell proliferation by deacetylating a number of additional protein substrates. For example, HDAC1-3 interact with the tumor suppressor gene p53 through the Sin3 complex, and decrease its stability through deacetylation [14]. In summary, due to the large panel of cell cycle regulatory proteins regulated by HDACs at the level of either their expression or activity, the anti-proliferative effect of HDAC inhibitors cannot be linked to a single mechanism of action.

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Compound screening campaigns followed by extensive structure–activity relationship studies revealed compound **1** as a new highly potent HDAC inhibitor (Fig. 1) [15]. Compound **1** fits the proposed general pharmacophore model for HDAC inhibitors [16–19] shown in Fig. 1, which consists of a 'capping region' linked to a 'enzyme inhibiting group' via a lipophilic linker. Presumably the naphtyl moiety interacts with the capping region while the hydroxamic acid chelates the active site zinc molecule in the enzyme.

During the course of developing potent HDAC inhibitors, which culminated with the discovery of compound **1** [20], we studied the impact of linker modifications on the inhibitory potency of HDAC activity in cell free assays. We wish to report here the synthesis and HDAC inhibiting activity of several amino-heterocyclic analogs of compound **1**.

2. Chemistry

Compounds **7a–d** were prepared according to the general procedure described in Scheme 1. Sulfonamides **3a–d** [21] were reacted with 2-methylsulfonyl-5-pyrimidinecarboxylic acid ethyl ester (**2**) [22] in basic medium to provide esters **4a–d** with good yields. The esters **4a–d** were saponified into acids **5a–d**, isolated as their sodium salts which were coupled with protected hydroxylamine to give amides **6a–d**. Then, removal of the tetrahydropyranyl (THP) protecting moiety in acidic conditions afforded hydroxamic acids **7a–d**.

Synthesis of 4-aminomethylpiperidinyl analog **14** (Scheme 2) illustrates that the sequence of reactant introduction can be varied and adapted to available starting material. Commercially available 4-aminomethyl-1-piperidine carboxylic acid-1,1-dimethylester was reacted with **2** to give ester **9**. After removal of the Boc-protection, 2-naphtylsulfonyl chloride was condensed on piperidinyl nitrogen to provide sulfonylamide **11**. Subsequently, hydroxamic acid was introduced as described in Scheme 1 to afford final compound **14**.

The synthesis of pyridinyl analogs **19a–b** (Scheme 3) followed a similar pathway as described in Scheme 1 except that **2** was now replaced in the first step by commercially available 2-chloronicotinic acid ethyl ester **15**.

3. Results and discussion

Inhibitory potency of these new compounds on HDAC activity was evaluated using HeLa cell nuclear extracts as

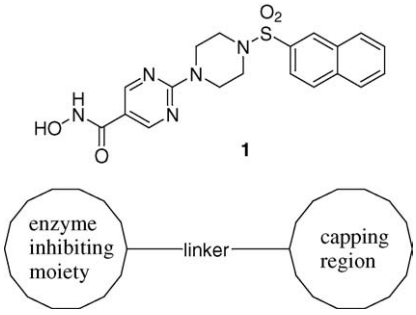
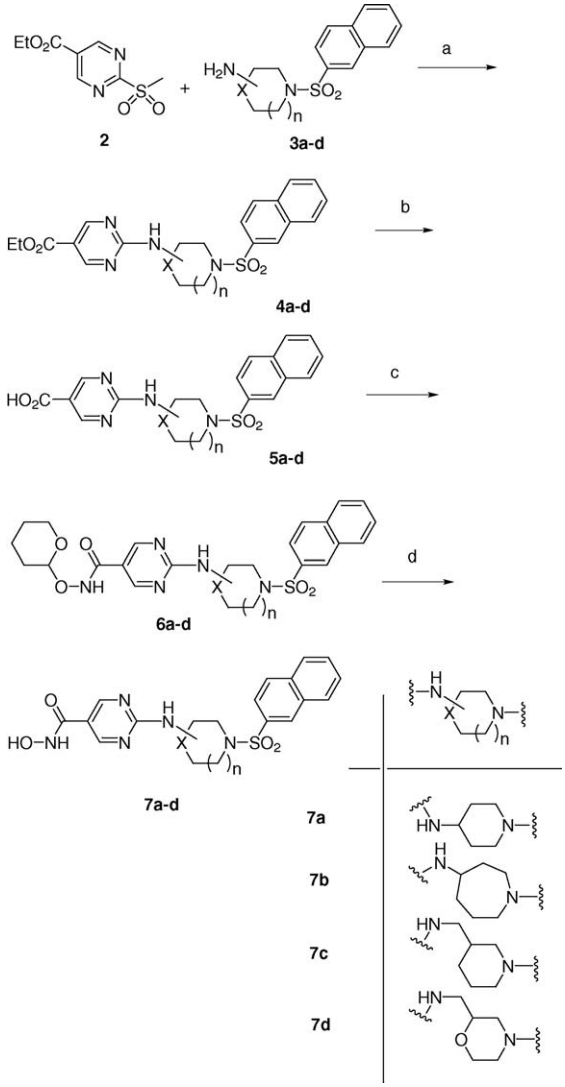


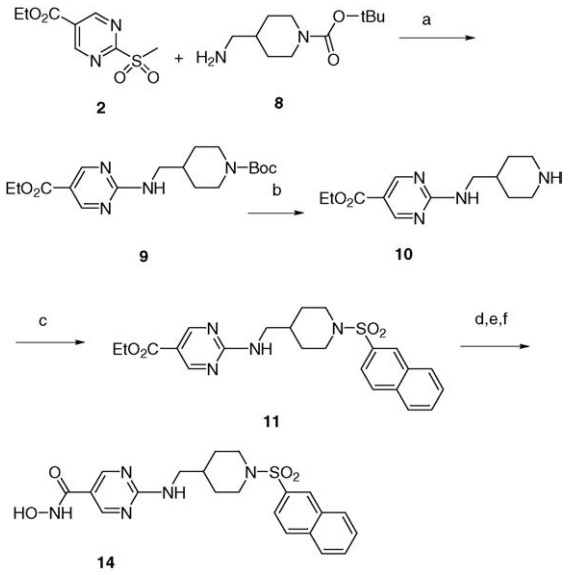
Fig. 1. Structure of HDAC inhibitor and HDAC pharmacophore model.



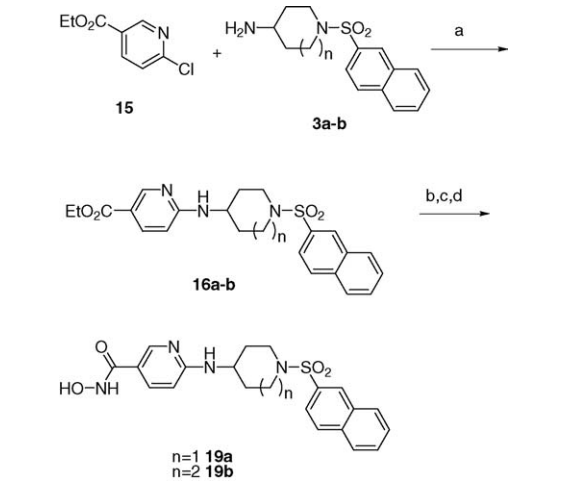
Scheme 1. (a) NaH, THF, RT, 12 h (34–39%, **4a–c**) or K_2CO_3, CH_3CN, RT, 12 h (89%, **4d**); (b) NaOH, EtOH, 80 °C, 2 h; (c) H_2N–O–THP, EDC, HOBT, THF, RT, 18 h (24–68%); (d) CF_3COOH, MeOH, RT (26–75%).

enzyme source and a radiolabeled acetylated histone four peptide fragment as substrate. In routinely running dose-response studies, all compounds showed inhibition of HDAC with IC_{50} values below or around 100 nM. Subsequently their antiproliferative potency was assessed using human A2780 ovarian carcinoma cells. These cells were seeded at low density and were incubated after 24 h with different concentrations of the tested compounds. The number of viable cells after a 4-day incubation period was determined using a standard MTT colorimetric assay. The results are reported in Table 1.

The 4-aminopiperidinyl analog **7a** was found to be a potent inhibitor of HDAC activity, with an IC_{50} value of 30 nM. However, its activity on A2780 cell proliferation as compared to compound **1** showed a 70-fold decrease. Increasing the cycle size to a seven membered ring (homopiperidinyl analog **7b**) maintained the potency whereas introduction of a methyl group between the amino moiety and the piperidine in **14** lead to a twofold decrease in cell proliferation. We then investigated the effect of 3-aminomethylpiperidinyl analog



Scheme 2. (a) NaH, THF, RT, 2 h (35%); (b) HCl 3 N, THF, 80 °C, 6 h (33%); (c) 2-naphtylsulfonylchloride, NEt_3, CH_2Cl_2, RT, 12 h (80%); (d) NaOH, EtOH, 80 °C, 12 h (81%); (e) H_2N–O–THP, EDC, HOBT, THF, RT, 18 h (90%); (f) CF_3COOH, MeOH, RT, 8d (33%).



Scheme 3. (a) Na_2CO_3, DMA, 130 °C, 18 h (27–43%); (b) NaOH, EtOH, 80 °C, 2 h (82%-quant.); (c) H_2N–O–THP, EDC, HOBT, THF, RT, 18 h (42–77%); (d) CF_3COOH, MeOH, RT (18–48%).

Table 1
HDAC and anti-proliferative inhibiting potency of compounds **7a**–**d**, **14** and **19a**–**b**

Compound number	IC_{50} enzyme (nM)[a]	IC_{50} cells (μM)[b]
1	8	0.05
7a	30	3.4
7b	49	3.1
7c	59	1.9
7d	69	2.2
14	39	7.8
19a	21	2.5
19b	116	3.5

[a] Radioactivity based HDAC assay (see Section 5).
[b] The concentration of the drug needed to reduce cell growth to 50% of the control.

7c and the 3-aminomethylmorpholinyl analog **7d**. Their enzymatic activity suffered from a twofold decrease as compared to **7a** but their anti-proliferative effect was slightly better. Replacing the pyrimidinyl ring by a pyridinyl moiety gave one of the best compounds of the series (**19a**). As previously noted for the pyrimidinyl series, introduction of the homopiperidinyl heterocycle led in **19b** to a drop in enzymatic activity whereas the anti-proliferative potency remained equal.

4. Conclusion

We have shown that amino-2-pyrimidinyl can be used as a linker to provide HDAC inhibitors of good enzymatic potency. Unfortunately the anti-proliferative potency on A2780 cells of such compounds remained in the micromolar range, which represents a 40-fold decrease as compared with lead compound **1**. Further modifications are being studied in order to identify compounds with a better cellular potency. These results will be reported on due course.

5. Experimental

5.1. Chemistry

Proton NMR spectra were recorded at 400 MHz or at 300 MHz on a Bruker Avance 400, or 300 on a Bruker spectrometer, with Me_4Si as internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Coupling constant are given in hertz (Hz). Electrospray mass spectra were recorded on an Waters/Micromass LCT spectrometer. Melting points (m.p.) were determined on a Mettler Toledo FP62 apparatus and are uncorrected. All reactions were routinely checked by TLC on silica gel Merck 60F 254. Column chromatography was carried out on Millipore silica gel (25–45 μM).

*5.1.1. General procedure for the synthesis of 2-[1-(naphthalene-2-sulfonyl)-heterocyclyl]-pyrimidine-5-carboxylic acid ethyl ester (**4a**–**c**)*

Sodium hydride 60% in oil (0.0045 mol) was added at 5 °C to a mixture of sulfonylamide **3** (0.003 mol) in THF (20 ml) under N_2 flow. The mixture was kept for 1 h. A solution of 2-(methylsulfonyl)-5-pyrimidinecarboxylic acid, ethyl ester (0.0039 mol) in THF (10 ml) was added. The mixture was stirred at 5 °C for 2 h. Water was added. The mixture was extracted twice with CH_2Cl_2 (DCM). The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue was purified by column chromatography over silica gel (15–40 μm) (eluent: DCM/MeOH 99:1). The pure fractions were collected and the solvent was evaporated to afford 34–39% of **4a**–**c**.

*5.1.1.1. 2-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-pyrimidine-5-carboxylic acid ethyl ester (**4a**).* Recrystallized from diethyl ether. M.p. = 226 °C, ^{1}H-NMR (300 MHz,

[D_6]DMSO, 27 °C): δ = 1.25 (t, J = 7.2 Hz, 3H, $CH_{3\text{-ester}}$), 1.50–1.66 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.85–1.96 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.52–2.64 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.57–3.67 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.72–3.86 (m, 1H, $H_{4\text{-piperidinyl}}$), 4.23 (q, J = 7.2 Hz, 2H, $CH_{2\text{-ester}}$), 7.65–7.80 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.06–8.25 (m, 4H, $H_{4,5,8\text{-naphtyl}}$, –NH), 8.44 (s, 1H, $H_{1\text{-naphtyl}}$), 8.67 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 13.0 (**C**H_3–CH_2), 29.0 (2C, $C_{3,5\text{-piperidinyl}}$), 43.6 (2C, $C_{2,6\text{-piperidinyl}}$), 45.4 ($C_{4\text{-piperidinyl}}$), 59.0 ($CH_3$–**C**$H_2$), 111.2 ($C_{5\text{-pyrimidinyl}}$), 121.7 ($C_{3\text{-naphtyl}}$), 126.5 ($C_{7\text{-naphtyl}}$), 126.7 ($C_{5\text{-naphtyl}}$), 127.4 ($C_{1\text{-naphtyl}}$), 127.8 ($C_{6\text{-naphtyl}}$), 128.2 (2C, $C_{8,4\text{-naphtyl}}$), 130.7 ($C_{9\text{-naphtyl}}$), 131.8 ($C_{2\text{-naphtyl}}$), 133.3 ($C_{10\text{-naphtyl}}$), 158.3 (2C, $C_{4,6\text{-pyrimidinyl}}$), 161.3 ($C_{2\text{-pyrimidinyl}}$), 162.9 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{22}H_{24}N_4O_4S$ 441.1596, found 441.1602.

*5.1.1.2. 2-[1-(Naphthalene-2-sulfonyl)-azepan-4-ylamino]-pyrimidine-5-carboxylic acid ethyl ester (**4b**).* Crystallized from diethyl ether. M.p. = 186 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.28 (t, J = 7.1 Hz, 3H, $CH_{3\text{-ester}}$), 1.55–2.08 (m, 6H, $CH_{2\text{-homopiperidinyl}}$), 3.15–3.55 (m, 4H), 3.92–4.03 (m, 1H, $CH_{\text{homopiperidinyl}}$), 4.25 (q, J = 7.1 Hz, 2H, $CH_{2\text{-ester}}$), 7.66–7.74 (m, 2H), 7.80 (d, J = 8.6 Hz, 1H), 8.07 (d, J = 8.6 Hz, 1H), 8.11–8.21 (m, 3H), 8.50 (s, 1H), 8.68–8.75 (m, 2H, $CH_{\text{pyrimidinyl}}$) ppm. LRMS (ESI), calc. for $C_{23}H_{26}N_4O_4S$ 454.17, found 455.2[MH]$^+$.

*5.1.1.3. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-3-ylmethyl]-amino}-pyrimidine-5 carboxylic acid ethyl ester (**4c**).* ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 0.88–1.00 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.30 (t, J = 7.1 Hz, 3H, C**H**$_3$–CH_2–), 1.40–1.51 (m, 1H, $H_{5\text{-piperidinyl}}$), 1.57–1.73 (m, 2H, $H_{4,5\text{-piperidinyl}}$), 1.78–1.90 (m, 1H, $H_{3\text{-piperidinyl}}$), 2.10 (t, J = 10.9 Hz, 1H, $H_{2\text{-piperidinyl}}$), 2.34 (td, J = 10.9 Hz and 2.3 Hz, 1H, $H_{6\text{-piperidinyl}}$), 3.10–3.32 (m, 2H, NH–C**H**$_2$), 3.54–3.60 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.60–3.66 (m, 1H, $H_{2\text{-piperidinyl}}$), 4.29 (q, J = 7.1 Hz, 2H, CH_3–C**H**$_2$–), 7.65–7.77 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.08 (d, J = 8.1 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.12–8.18 (m, 2H), 8.25 (t, J = 6.0 Hz, 1H, CH_2–N**H**), 8.40 (s, 1H, $H_{1\text{-naphtyl}}$), 8.69–8.74 (m, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 14.4 (**C**H_3–CH_2–), 23.9 ($C_{5\text{-piperidinyl}}$), 27.0 ($C_{4\text{-piperidinyl}}$), 35.6 ($C_{3\text{-piperidinyl}}$), 43.9 (NH–**C**H_2), 46.7 ($C_{6\text{-piperidinyl}}$), 50.0 ($C_{2\text{-piperidinyl}}$), 60.4 (CH_3–**C**H_2–), 112.6 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 127.8 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.2 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4,8\text{-naphtyl}}$), 132.0 (1C), 133.0 (1C), 134.6 ($C_{10\text{-naphtyl}}$), 159.7 (2C, $C_{4,6\text{-pyrimidinyl}}$), 163.6 ($C_{2\text{-pyrimidinyl}}$), 164.3 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{23}H_{26}N_4O_4S$ 455.1753, found 455.1752.

*5.1.2. 2-{[4-(Naphthalene-2-sulfonyl)-morpholin-2-ylmethyl]-amino}-pyrimidine-5-carboxylic acid ethyl ester (**4d**)*

A mixture of C-[4-(naphthalene-2-sulfonyl)-morpholin-2-yl]-methylamine [21] (0.0019 mol), 2-(methylsulfonyl)-5-pyrimidinecarboxylic acid, ethyl ester (0.0025 mol) and potassium carbonate (0.0039 mol) in acetonitrile (15 ml) was stirred at room temperature for 12 h, poured out into ice water and extracted with EtOAc. The organic layer was washed with water, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (1 g) was purified by column chromatography over silica gel (15–40 μm) (eluent: DCM/EtOAc 80:20). The pure fractions were collected and the solvent was evaporated. The residue (0.48 g, 89%) was crystallized from CH_3CN/diethyl ether. The precipitate was filtered off and dried, yielding 0.2 g of **4d**. M.p. = 168 °C. ^{1}H-NMR (300 MHz, [D_6] DMSO, 27 °C): δ = 1.31 (t, J = 7.1 Hz, 3H, –CH_2–C**H**$_3$), 2.11 (t, J = 11.3 Hz, 1H, $H_{3\text{-morpholinyl}}$), 2.38 (td, J = 12.0 Hz and 3.0 Hz, 1H, $H_{5\text{-morpholinyl}}$), 3.22–3.38 (m, 1H, NH–C**H**$_2$), 3.40–3.58 (m, 3H, NH–C**H**$_2$, $H_{5,6\text{-morpholinyl}}$), 3.58–3.73 (m, 2H, $H_{2,3\text{-morpholinyl}}$), 3.80–3.94 (m, 1H, $H_{6\text{-morpholinyl}}$), 4.29 (q, J = 7.0 Hz, 2H, CH_3–C**H**$_2$), 7.64–7.81 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.12–8.24 (m, 3H, CH_2–N**H**, $H_{4,8\text{-naphtyl}}$), 8.40 (s, 1H, $H_{1\text{-naphtyl}}$), 8.69 (s, 1H, $H_{\text{pyrimidinyl}}$), 8.74 (s, 1H, $H_{\text{pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 14.6 (**C**H_3–CH_2), 43.2 (NH–**C**H_2), 45.8 ($C_{\text{morpholinyl}}$), 48.9 ($C_{\text{morpholinyl}}$), 60.6 (CH_3–**C**H_2), 65.4 ($C_{6\text{-morpholinyl}}$), 73.4 ($C_{2\text{-morpholinyl}}$), 113.0 ($C_{5\text{-pyrimidinyl}}$), 123.2 ($C_{3\text{-naphtyl}}$), 128.1 ($C_{7\text{-naphtyl}}$), 128.2 ($C_{5\text{-naphtyl}}$), 129.3 ($C_{1\text{-naphtyl}}$), 129.5 ($C_{6\text{-naphtyl}}$), 129.7 (C_{naphtyl}), 129.8 (C_{naphtyl}), 132.1 ($C_{9\text{-naphtyl}}$), 132.1 ($C_{2\text{-naphtyl}}$), 134.9 ($C_{10\text{-naphtyl}}$), 159.9 (2C, $C_{4,6\text{-pyrimidinyl}}$), 163.6 ($C_{2\text{-pyrimidinyl}}$), 164.4 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{22}H_{24}N_4O_5S$ 457.1545, found 457.1533.

*5.1.3. General procedure for the synthesis of 2-[1-(naphthalene-2-sulfonyl)-heterocyclyl]-pyrimidine-5-carboxylic acids sodium salts (**5a–d**)*

A mixture of ester **4a–d** (0.0014 mol) and sodium hydroxide (0.0028 mol) in EtOH (10 ml) was stirred and refluxed for 2–3 h, then cooled. The precipitate was filtered, washed with EtOH, then with diethyl ether and dried, yielding **5a–d**. These compounds were used without further purification in the next step.

*5.1.3.1. 2-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-pyrimidine-5-carboxylic acid (**5a**).* Yield = quant., ^{1}H-NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.48–1.53 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.85–1.96 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.53–2.65 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.54–3.79 (m, 3H, $H_{2,4,6\text{-piperidinyl}}$), 7.18 (d, J = 7.5 Hz, 1H, –NH), 7.65–7.82 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.18 (d, J = 8.7 Hz, 1H, $H_{4\text{-naphtyl}}$), 8.22 (d, J = 7.9 Hz, 1H, $H_{8\text{-naphtyl}}$), 8.44 (s, 1H, $H_{1\text{-naphtyl}}$), 8.55 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 30.7 (2C, $C_{3,5\text{-piperidinyl}}$), 45.1 (2C, $C_{2,6\text{-piperidinyl}}$), 46.5 ($C_{4\text{-piperidinyl}}$), 121.9 ($C_{5\text{-pyrimidinyl}}$), 123.1 ($C_{3\text{-naphtyl}}$), 127.8 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.1 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4,8\text{-naphtyl}}$), 132.1 ($C_{9\text{-naphtyl}}$), 133.1 ($C_{2\text{-naphtyl}}$), 134.6 ($C_{10\text{-naphtyl}}$), 159.2 (2C, $C_{4,6\text{-pyrimidinyl}}$), 161.7 ($C_{2\text{-pyrimidinyl}}$), 167.5 (COONa) ppm. HRMS (ESI), calc. for $C_{20}H_{20}N_4O_4S$ 413.1283, found 413.1292.

*5.1.3.2. 2-[1-(Naphthalene-2-sulfonyl)-azepan-4-ylamino]-pyrimidine-5-carboxylic acid (**5b**).* Yield = 93%, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 0.82–0.96 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.39–1.51 (m, 1H, $H_{5\text{-piperidinyl}}$), 1.58–1.63 (m, 2H, $H_{4,5\text{-piperidinyl}}$), 1.63–1.86 (m, 1H, $H_{3\text{-piperidinyl}}$), 2.03 (dd, J = 10.9 Hz, 1H, $H_{2\text{-piperidinyl}}$), 2.21–2.31 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.00 (dd, J = 13.6 Hz and 7.8 Hz, 1H, NH–C**H**$_2$), 3.15 (dd, J = 13.6 Hz and 5.5 Hz, 1H, NH–C**H**$_2$), 3.54–3.62 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.67–3.74 (m, 1H, $H_{2\text{-piperidinyl}}$), 7.65–7.77 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.08 (d, J = 8.1 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.15 (d, J = 9.1 Hz, 1 Hz, $H_{4\text{-naphtyl}}$), 8.20 (d, J = 8.1 Hz, 1H, $H_{8\text{-naphtyl}}$), 8.36 (s, 1H, $H_{1\text{-naphtyl}}$), 8.50 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 24.2 ($C_{5\text{-piperidinyl}}$), 27.5 ($C_{4\text{-piperidinyl}}$), 36.4 ($C_{3\text{-piperidinyl}}$), 45.2 (NH–**C**H$_2$), 46.8 ($C_{6\text{-piperidinyl}}$), 50.6 ($C_{2\text{-piperidinyl}}$), 122.4 ($C_{5\text{-pyrimidinyl}}$), 123.1 ($C_{3\text{-naphtyl}}$), 127.9 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.6 ($C_{1\text{-naphtyl}}$), 129.1 ($C_{6\text{-naphtyl}}$), 129.4 (C_{naphtyl}), 129.6 (C_{naphtyl}), 132.0 (C_{naphtyl}), 132.9 (C_{naphtyl}), 134.6 ($C_{10\text{-naphtyl}}$), 159.3 (2C, $C_{4,6\text{-pyrimidinyl}}$), 163.3 ($C_{2\text{-pyrimidinyl}}$), 168.6 (COONa) ppm. HRMS (ESI), calc. for $C_{21}H_{22}N_4O_4S$ 427.1440, found 427.1448.

*5.1.3.3. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-3-ylmethyl]-amino}-pyrimidine-5-carboxylic acid sodium salt (**5c**).* Yield = 83%, LRMS (ESI), calc. for $C_{21}H_{22}N_4O_4S$ 426.5, found 427.3[MH]$^+$.

*5.1.3.4. 2-{[4-(Naphthalene-2-sulfonyl)-morpholin-2-ylmethyl]-amino}-pyrimidine-5-carboxylic acid sodium salt (**5d**).* Yield = quant., ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 2.09 (t, J = 11.3 Hz, 1H, $H_{3\text{-morpholinyl}}$), 2.28–2.45 (m, 1H, $H_{5\text{-morpholinyl}}$), 3.17–3.30 (m, 1H, NH–C**H**$_2$), 3.35–3.58 (m, 3H, NH–C**H**$_2$, $H_{5,6\text{-morpholinyl}}$), 3.60–3.70 (m, 2H, $H_{2,3\text{-morpholinyl}}$), 3.82–3.92 (m, 1H, $H_{6\text{-morpholinyl}}$), 7.25 (t, J = 6.2 Hz, 1H, CH_2–N**H**), 7.67–7.80 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.15–8.22 (m, 2H, H_{naphtyl}), 8.38 (s, 1H, $H_{10\text{-naphtyl}}$), 8.65 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 43.1 (NH–**C**H$_2$), 45.7 ($C_{5\text{-morpholinyl}}$), 49.0 ($C_{3\text{-morpholinyl}}$), 65.3 ($C_{6\text{-morpholinyl}}$), 73.6 ($C_{2\text{-morpholinyl}}$), 122.2 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 128.0 (2C, $C_{5,7\text{-naphtyl}}$), 129.1 ($C_{1\text{-naphtyl}}$), 129.4 ($C_{6\text{-naphtyl}}$), 129.6 (2C, $C_{4,8\text{-naphtyl}}$), 131.9 (2C, $C_{2,9\text{-naphtyl}}$), 134.7 ($C_{10\text{-naphtyl}}$), 159.4 (2C, $C_{4,6\text{-pyrimidinyl}}$), 162.3 ($C_{2\text{-pyrimidinyl}}$), 167.6 (COONa) ppm. HRMS (ESI), calc. for $C_{20}H_{20}N_4O_5S$ 429.1233, found 429.1235.

*5.1.4. General procedure for the synthesis of 2-[1-(naphthalene-2-sulfonyl)-heterocyclyl]-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amides (**6a**–**d**)*

N′-(ethylcarbonimidoyl)-*N*,*N*-dimethyl-1,3-propanediamine, monohydrochloride (0.0013 mol) was added to a mixture of compounds **5a**–**d** (0.0011 mol), O-(tetrahydro-2H-pyran-2-yl)hydroxylamine (0.0013 mol) and 1-hydroxy-1H-benzotriazole (0.0013 mol) in DCM/THF (10 ml) under N_2 flow. The mixture was stirred at room temperature overnight. Potassium carbonate 10% was added. The mixture was extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: DCM/MeOH/NH_4OH 97:3:0.2). The pure fractions were collected and the solvent was evaporated yielding (24–68%) of **6a**–**d**.

*5.1.4.1. 2-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amide (**6a**).* Yield = 24%. ^{1}H-NMR (300 MHz, $CDCl_3$, 27 °C): δ = 1.47–1.80 (m, 8H, $H_{3,4,5\text{-tetrahydropyranyl}}$, $H_{3,5\text{-piperidinyl}}$), 2.00–2.15 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.53–2.68 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.55–3.71 (m, 2H, $H_{6\text{-tetrahydropyranyl}}$), 3.73–3.88 (m, 3H, $H_{2,6\text{-piperidinyl}}$, $H_{4\text{-piperidinyl}}$), 3.90–4.04 (m, 2H, $H_{6\text{-tetrahydropyranyl}}$), 4.96–5.04 (m, 1H, $H_{2\text{-tetrahydropyranyl}}$), 5.78 (d, J = 7.5 Hz, 1H, –NH), 7.58–7.72 (m, 2H, $H_{6,7\text{-naphtyl}}$), 7.76 (d, J = 8.3 Hz, 1H, $H_{3\text{-naphtyl}}$), 7.90–8.04 (m, 3H, $H_{4,5,8\text{-naphtyl}}$), 8.34 (s, 1H, $H_{1\text{-naphtyl}}$), 8.59 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 9.24 (br s, 1H, CO–NH–O) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$, 27 °C): δ = 18.6, 25.1, 28.1 ($C_{3,4,5\text{-tetrahydropyranyl}}$), 31.2 (2C, $C_{3,5\text{-piperidinyl}}$), 45.2 (2C, $C_{2,6\text{-piperidinyl}}$), 47.5 ($C_{4\text{-piperidinyl}}$), 62.7 ($C_{6\text{-tetrahydropyranyl}}$), 102.9 ($C_{2\text{-tetrahydropyranyl}}$), 115.3 ($C_{5\text{-pyrimidinyl}}$), 122.8 ($C_{3\text{-naphtyl}}$), 127.7 ($C_{7\text{-naphtyl}}$), 128.0 ($C_{5\text{-naphtyl}}$), 128.9, 129.0, 129.2, 129.3 ($C_{1,4,6,8\text{-naphtyl}}$), 132.2 ($C_{9\text{-naphtyl}}$), 133.0 ($C_{2\text{-naphtyl}}$), 134.9 ($C_{10\text{-naphtyl}}$), 158.0 (2C, $C_{4,6\text{-pyrimidinyl}}$), 162.2 (C=O) ppm. HRMS (ESI), calc. for $C_{25}H_{29}N_5O_5S$ 512.1967, found 512.1946.

*5.1.4.2. 2-[1-(Naphthalene-2-sulfonyl)-azepan-4-ylamino]-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amide (**6b**).* Yield = 39%. M.p. = 185 °C. ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.50–2.00 (m, 12H, $CH_{2\text{-tetrahydropyranyl}}$ + $CH_{2\text{-homopiperidinyl}}$), 3.15–3.50 (m, 5H), 3.90–4.18 (m, 2H), 4.95–4.98 (m, 1H, $OCH_{\text{tetrahydropyranyl}}$), 7.66–7.90 (m, 4H, $3H_{\text{naphtyl}}$ + $NH_{\text{homopiperidinyl}}$), 8.07 (d, J = 8.1 Hz, 1H, H_{naphtyl}), 8.15 (d, J = 8.6 Hz, 1H, H_{naphtyl}), 8.20 (d, J = 8.1 Hz, 1H, H_{naphtyl}), 8.47 (s, 1H), 8.60 (s, 2H, $H_{\text{pyrimidinyl}}$), 11.46 (s, 1H, N**H**–O–THP) ppm. LRMS (ESI), calc. for $C_{26}H_{31}N_5O_5S$ 525.6, found 526.3[MH]$^+$.

*5.1.4.3. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-3-ylmethyl]-amino}-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amide (**6c**).* Yield = 68%, ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 0.85–1.03 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.37–1.80 (m, 10H, $H_{3,4,5\text{-piperidinyl}}$, $H_{3,4,5\text{-tetrahydropyranyl}}$), 2.03–2.15 (m, 1H, $H_{2\text{-piperidinyl}}$), 2.27–2.38 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.05–3.32 (m, 2H, NH–C**H**$_2$), 3.21–3.37 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.50–3.60 (m, 2H, $H_{6\text{-tetrahydropyranyl}}$), 3.65 (dd, J = 10.9 Hz and 2.6 Hz, 1 Hz, $H_{2\text{-piperidinyl}}$), 4.00–4.10 (m, 1H, $H_{6\text{-tetrahydropyranyl}}$), 4.95–5.00 (m, 1H, $H_{2\text{-tetrahydropyranyl}}$), 7.65–7.77 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 7.97 (t, J = 6.0 Hz, 1H, CH_2–N**H**), 8.08 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.12–8.18 (m, 2H, H_{naphtyl}), 8.38 (s, 1H, $H_{1\text{-naphtyl}}$), 8.61 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 11.50 (s, 1H, CO–NH–O) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 18.6 ($C_{4\text{-tetrahydropyranyl}}$), 24.0

($C_{5\text{-piperidinyl}}$), 24.9 ($C_{5\text{-tetrahydropyranyl}}$), 27.1 ($C_{4\text{-piperidinyl}}$), 28.1 ($C_{3\text{-tetrahydropyranyl}}$), 35.7 ($C_{3\text{-piperidinyl}}$), 43.8 (NH–**C**$H_2$), 46.7 ($C_{6\text{-piperidinyl}}$), 50.0 ($C_{2\text{-piperidinyl}}$), 61.7 ($C_{6\text{-tetrahydropyranyl}}$), 101.5 ($C_{2\text{-tetrahydropyranyl}}$), 114.6 ($C_{5\text{-pyrimidinyl}}$), 123.1 ($C_{3\text{-naphtyl}}$), 127.9 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.2 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4,8\text{-naphtyl}}$), 132.0 (C_{naphtyl}), 133.0 (C_{naphtyl}), 134.6 ($C_{10\text{-naphtyl}}$), 157.7 (2C, $C_{4,6\text{-pyrimidinyl}}$), 162.4 (C=O), 163.2 ($C_{2\text{-pyrimidinyl}}$) ppm. HRMS (ESI), calc. for $C_{26}H_{31}N_5O_5S$ 526.2124, found 526.2122.

*5.1.4.4. 2-{[4-(Naphthalene-2-sulfonyl)-morpholin-2-ylmethyl]-amino}-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amide (**6d**).* Yield = 29%, ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.46–1.64 (m, 3H, $H_{3,4,5\text{-tetrahydropyranyl}}$), 1.66–1.81 (m, 3H, $H_{3,4,5\text{-tetrahydropyranyl}}$), 2.10 (t, J = 11.3 Hz, 1H, $H_{3\text{-morpholinyl}}$), 2.85 (td, J = 11.9 Hz and 2.6 Hz, 1H, $H_{5\text{-morpholinyl}}$), 3.20–3.36 (m, 1H, NH–C**H**$_2$), 3.38–3.46 (m, 1H, NH–C**H**$_2$), 3.46–3.59 (m, 2H, $H_{5,6\text{-morpholinyl}}$), 3.59–3.69 (m, 2H, $H_{2,3\text{-morpholinyl}}$), 3.81–3.92 (m, 1H, $H_{6\text{-morpholinyl}}$), 4.95–5.03 (m, 1H, $C_{2\text{-tetrahydropyranyl}}$), 7.66–7.80 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 7.90 (t, J = 6.0 Hz, 1H, CH_2–N**H**), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.13–8.21 (m, 2H, $H_{4,8\text{-naphtyl}}$), 8.40 (s, 1H, $H_{1\text{-naphtyl}}$), 8.56–8.70 (m, 2H, $H_{4,6\text{-pyrimidinyl}}$), 11.53 (s, 1H, CO–NH–O) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 18.6 ($C_{4\text{-tetrahydropyranyl}}$), 24.9 ($C_{\text{tetrahydropyranyl}}$), 28.1 ($C_{\text{tetrahydropyranyl}}$), 43.0 (NH–**C**$H_2$), 45.7 ($C_{5\text{-morpholinyl}}$), 48.7 ($C_{3\text{-morpholinyl}}$), 61.7 ($C_{6\text{-tetrahydropyranyl}}$), 65.3 ($C_{6\text{-morpholinyl}}$), 73.4 ($C_{2\text{-morpholinyl}}$), 101.5 ($C_{2\text{-tetrahydropyranyl}}$), 114.9 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 128.0 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 129.1 ($C_{1\text{-naphtyl}}$), 129.4 ($C_{6\text{-naphtyl}}$), 129.6 (2C, $C_{4,8\text{-naphtyl}}$), 131.9 (2C, $C_{2,9\text{-naphtyl}}$), 134.7 ($C_{10\text{-naphtyl}}$), 157.6 ($C_{\text{pyrimidinyl}}$), 157.9 ($C_{\text{pyrimidinyl}}$), 162.3 ($C_{2\text{-pyrimidinyl}}$), 163.1 (C=O) ppm. HRMS (ESI), calc. for $C_{25}H_{29}N_5O_6S$ 528.1917, found 528.1891.

*5.1.5. General procedure for the synthesis of 2-[1-(naphthalene-2-sulfonyl)-heterocyclyl]-pyrimidine-5-carboxylic acid hydroxyamide (**7a–d**)*

Trifluoroacetic acid (0.5 ml) was added to a mixture of **6a–d** (0.0007 mol) in MeOH (5 ml). The mixture was stirred at room temperature for 18 h to 8 days. The solvent was evaporated till dryness. The residue was crystallized from MeOH/DCM/diethyl ether. The precipitate was filtered off and dried, yielding (26–75%) of **7a–d**.

*5.1.5.1. 2-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-pyrimidine-5-carboxylic acid hydroxyamide (**7a**).* Yield = 49%. M.p. = 256 °C, ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.48–1.65 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.83–1.98 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.53–2.65 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.55–3.67 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.69–3.83 (m, 1H, $H_{4\text{-piperidinyl}}$), 7.65–7.83 (m, 4H, $H_{3,6,7\text{-naphtyl}}$, –NH), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.18 (d, J = 8.7 Hz, 1H, $H_{4\text{-naphtyl}}$), 8.21 (d, J = 7.9 Hz, 1H, $H_{8\text{-naphtyl}}$), 8.44 (s, 1H, $H_{1\text{-naphtyl}}$), 8.54 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 10.99 (s, 1H, N**H**–OH) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 30.7 (2C, $C_{3,5\text{-piperidinyl}}$), 45.2 (2C, $C_{2,6\text{-piperidinyl}}$), 46.8 ($C_{4\text{-piperidinyl}}$), 115.2 ($C_{5\text{-pyrimidinyl}}$), 123.2 ($C_{3\text{-naphtyl}}$), 128.0 ($C_{7\text{-naphtyl}}$), 128.2 ($C_{5\text{-naphtyl}}$), 128.9 ($C_{1\text{-naphtyl}}$), 129.3 ($C_{6\text{-naphtyl}}$), 129.7 (2C, $C_{4,8\text{-naphtyl}}$), 132.2 ($C_{9\text{-naphtyl}}$), 133.3 ($C_{2\text{-naphtyl}}$), 134.8 ($C_{10\text{-naphtyl}}$), 157.4 (2C, $C_{4,6\text{-pyrimidinyl}}$), 162.3 (C=O) ppm. HRMS (ESI), calc. for $C_{20}H_{21}N_5O_4S$ 428.1393, found 428.1391.

*5.1.5.2. 2-[1-(Naphthalene-2-sulfonyl)-azepan-4-ylamino]-pyrimidine-5-carboxylic acid hydroxyamide (**7b**).* Yield = 26%. M.p. = 140 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.56–2.00 (m, 6H, $H_{\text{homopiperidinyl}}$), 3.20–3.55 (m, 4H, $H_{\text{homopiperidinyl}}$), 3.90–3.98 (m, 1H, $H_{\text{homopiperidinyl}}$), 7.70–7.80 (m, 4H, $3H_{\text{naphtyl}}$ + $NH_{\text{homopiperidinyl}}$), 8.00–8.20 (m, 3H, H_{naphtyl}), 8.47 (s, 1H, H_{naphtyl}), 8.58 (s, 2H, $H_{\text{pyrimidinyl}}$), 9.00 (br s, 1H), 10.86 (s, 1H) ppm. LRMS (ESI), calc. for $C_{21}H_{23}N_5O_4S$ 441.5, found 442.2[MH^+].

*5.1.5.3. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-3-ylmethyl]-amino}-pyrimidine-5-carboxylic acid hydroxyamide (**7c**).* Yield = 75%. M.p. = 137 °C, ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 0.85–1.00 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.37–1.54 (m, 1H, $H_{5\text{-piperidinyl}}$), 1.54–1.77 (m, 2H, $H_{4,5\text{-piperidinyl}}$), 1.75–1.95 (m, 1H, $H_{3\text{-piperidinyl}}$), 2.09 (t, J = 10.9 Hz, 1H, $H_{2\text{-piperidinyl}}$), 2.31 (td, J = 11.3 Hz and 1.9 Hz, 1H, $H_{6\text{-piperidinyl}}$), 3.00–3.15 (m, 1H, NH–C**H**$_2$), 3.20–3.35 (m, 1H, NH–C**H**$_2$), 3.53–3.61 (m, 1H, $H_{6\text{-piperidinyl}}$), 3.64 (dd, J = 11.3 Hz and 1.9 Hz, 1H, $H_{2\text{-piperidinyl}}$), 7.65–7.78 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 7.90 (t, J = 6.0 Hz, 1H, –CH_2–N**H**), 8.07 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.11–8.20 (m, 2H, H_{naphtyl}), 8.38 (s, 1H, $H_{1\text{-naphtyl}}$), 8.60 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 9.01 (br s, 1H, NH–O**H**), 11.05 (s, 1H, N**H**–OH) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 25 °C): δ = 24.0 ($C_{5\text{-piperidinyl}}$), 27.1 ($C_{4\text{-piperidinyl}}$), 35.6 ($C_{4\text{-piperidinyl}}$), 43.9 (NH–CH_2), 46.7 ($C_{6\text{-piperidinyl}}$), 50.0 ($C_{2\text{-piperidinyl}}$), 115.1 ($C_{5\text{-pyrimidinyl}}$), 123.1 ($C_{3\text{-naphtyl}}$), 127.9 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.2 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4,8\text{naphtyl}}$), 132.0 (C_{naphtyl}), 132.9 (C_{naphtyl}), 134.6 ($C_{10\text{-naphtyl}}$), 157.2 ($C_{\text{pyrimidinyl}}$), 157.6 ($C_{\text{pyrimidinyl}}$), 162.3 (C=O), 163.1 ($C_{2\text{-pyrimidinyl}}$) ppm. HRMS (ESI), calc. for $C_{21}H_{23}N_5O_4S$ 442.1549, found 442.1548.

*5.1.5.4. 2-{[4-(Naphthalene-2-sulfonyl)-morpholin-2-ylmethyl]-amino}-pyrimidine-5-carboxylic acid hydroxyamide (**7d**).* Yield = 73%. M.p. = 226 °C. ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 2.10 (t, J = 11.1 Hz, 1H, $H_{3\text{-morpholinyl}}$), 2.24–2.44 (m, 1H, $H_{5\text{-morpholinyl}}$), 3.14–3.32 (m, 1H, NH–C**H**$_2$), 3.32–3.45 (m, 1H, NH–C**H**$_2$), 3.45–3.58 (m, 2H, $H_{5,6\text{-morpholinyl}}$), 3.58–3.74 (m, 2H, $H_{2,3\text{-morpholinyl}}$), 3.78–3.94 (m, 1H, $H_{6\text{-morpholinyl}}$), 7.64–7.87 (m, 4H, $H_{3,6,7\text{-naphtyl}}$, CH_2–N**H**), 8.09 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.13–8.23 (m, 2H, $H_{4,8\text{-naphtyl}}$), 8.40 (s, 1H, $H_{1\text{-naphtyl}}$), 8.55–8.65 (m, 2H, $H_{4,6\text{-pyrimidinyl}}$), 9.03 (br s, 1H, OH), 11.07 (s, 1H, N**H**–OH) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 43.0

(NH–CH_2), 45.7 ($C_{5\text{-morpholinyl}}$), 48.8 ($C_{3\text{-morpholinyl}}$), 65.3 ($C_{6\text{-morpholinyl}}$), 73.4 ($C_{2\text{-morpholinyl}}$), 115.4 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 128.0 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 129.1 ($C_{1\text{-naphtyl}}$), 129.4 ($C_{6\text{-naphtyl}}$), 129.6 (2C, $C_{4,8\text{naphtyl}}$), 132.0 ($C_{2,9\text{-naphtyl}}$), 134.7 ($C_{10\text{-naphtyl}}$), 157.1 ($C_{\text{pyrimidinyl}}$), 157.7 ($C_{\text{pyrimidinyl}}$), 162.2 (C=O), 162.9 ($C_{2\text{-pyrimidinyl}}$) ppm. HRMS (ESI), calc. for $C_{20}H_{21}N_5O_5S$ 444.1342, found 444.1345.

*5.1.6. Synthesis of 2-[1-(naphthalene-2-sulfonyl)-[(piperidin-4-ylmethyl)-amino]]-pyrimidine-5-carboxylic acid hydroxyamide (**14**)*

*5.1.6.1. Preparation of 2-[(1-tert-butoxycarbonyl-piperidin-4-ylmethyl)-amino]-pyrimidine-5-carboxylic acid ethyl ester (**9**).* Sodium hydride 60% (0.008 mol) was added portionwise at 0 °C to a mixture of 4-(aminomethyl)-1-piperidinylcarboxylic acid-1,1-dimethylethyl ester (**8**) (0.004 mol) in THF (20 ml) under N_2 flow. The mixture was stirred at 0 °C for 1 h. A solution of 2-(methylsulfonyl)-5-pyrimidinecarboxylic acid ethyl ester (0.0052 mol) in THF (10 ml) was added dropwise at 0 °C. The mixture was brought to room temperature, stirred for 2 h, poured out into ice water and extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue (1.5 g) was purified by column chromatography over silica gel (15–40 μm) (eluent: DCM/MeOH/NH_4OH 98:2:0.1). The pure fractions were collected and the solvent was evaporated. The residue (0.5 g, 35%) was crystallized from DIPE. The precipitate was filtered off and dried, yielding 0.28 g of **9**. M.p. = 110 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 0.95–1.07 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.28 (t, J = 7.1 Hz, 3H, –CH_2–C**H**$_3$), 1.38 (s, 9H, $H_{t\text{-Butyl}}$), 1.51–1.79 (m, 3H, $H_{3,5\text{-piperidinyl}}$, $H_{4\text{-piperidinyl}}$), 2.53–2.80 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.24 (t, J = 6.1 Hz, 2H, C**H**$_2$–NH), 3.86–4.00 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 4.25 (q, J = 7.1 Hz, 2H, –C**H**$_2$–CH_3), 8.22 (t, J = 6.1 Hz, 1H, CH_2–N**H**), 8.68–8.70 (m, 1H, $H_{\text{pyrimidinyl}}$), 8.72–8.75 (m, 1H, $H_{\text{pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 14.4 (**C**H_3–CH_2–), 28.3 ((**C**H_3)$_3$–C), 29.7 (2C, $C_{3,5\text{-piperidinyl}}$), 35.6 ($C_{4\text{-piperidinyl}}$), 43.7 (2C, $C_{2,6\text{-piperidinyl}}$), 46.3 (NH–$CH_2$), 60.3 ($CH_3$–**C**$H_2$–), 78.6 (($CH_3$)$_3$–**C**), 112.3 ($C_{5\text{-pyrimidinyl}}$), 154.0 (O–CO–N), 159.7 ($C_{\text{pyrimidinyl}}$), 159.8 ($C_{4,6\text{-pyrimidinyl}}$), 163.7 ($C_{2\text{-pyrimidinyl}}$), 164.3 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{18}H_{28}N_4O_4S$ 365.2189, found 365.2174.

*5.1.6.2. 2-[(Piperidin-4-ylmethyl)-amino]-pyrimidine-5-carboxylic acid ethyl ester (**10**).* A mixture of **9** (0.016 mol) in hydrochloric acid 3 N (60 ml) and THF (15 ml) was stirred at 80 °C for 6 h, poured out into ice water, basified with NH_4OH and extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 1.4 g (33%) of **10**. ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 0.93–1.10 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.28 (t, J = 7.2 Hz, 3H, –CH_2–C**H**$_3$), 1.52–1.70 (m, 3H, $H_{3,5\text{-piperidinyl}}$, $H_{4\text{-piperidinyl}}$), 2.33–2.44 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 2.86–2.96 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.20 (t, J = 6.1 Hz, 2H, C**H**$_2$–NH), 4.25 (q, J = 7.2 Hz, 2H, –C**H**$_2$–CH_3), 8.15 (t, J = 6.1 Hz, 1H, CH_2–N**H**), 8.66–8.76 (m, 2H, $H_{\text{pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 14.6 (**C**H_3–CH_2), 31.2 (2C, $C_{3,5\text{-piperidinyl}}$), 36.3 ($C_{4\text{-piperidinyl}}$), 46.1 (2C, $C_{2,6\text{-piperidinyl}}$), 47.2 (NH–$CH_2$), 60.4 ($CH_3$–$CH_2$), 112.6 ($C_{5\text{-pyrimidinyl}}$), 159.8 ($C_{\text{pyrimidinyl}}$), 159.9 ($C_{\text{pyrimidinyl}}$), 163.8 ($C_{2\text{-pyrimidinyl}}$), 164.5 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{13}H_{20}N_4O_2$ 265.1664, found 265.1665.

*5.1.6.3. Ethyl-2-{[1-(naphthalene-2-sulfonyl)-piperidin-4-ylmethyl]-amino}-pyrimidine-5-carboxylate (**11**).* A solution of 2-naphthalenesulfonyl chloride (0.0006 mol) in DCM (2 ml) was added dropwise at 0 °C to a mixture of **10** (0.0005 mol) and triethylamine (0.0008 mol) in DCM (3 ml). The mixture was stirred at room temperature for 12 h, poured out into water and extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (10 μm) (eluent: DCM 100%). The pure fractions were collected and the solvent was evaporated, yielding 0.21 g (80%) of **11** .^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.12–1.29 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.26 (t, J = 7.2 Hz, 3H, –CH_2–C**H**$_3$), 1.45–1.60 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.68–1.78 (m, 1H, $H_{3,5\text{-piperidinyl}}$), 2.21–2.33 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.18 (t, J = 6.1 Hz, 2H, C**H**$_2$–NH), 3.65–3.75 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 4.23 (q, J = 7.2 Hz, 2H, –C**H**$_2$–CH_3), 7.64–7.77 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.04–8.23 (m, 4H, $H_{4,5,8\text{-naphtyl}}$, CH_2–N**H**), 8.42 (s, 1H, $H_{1\text{-naphtyl}}$), 8.65 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 14.4 (**C**H_3–CH_2–), 29.0 (2C, $C_{3,5\text{-piperidinyl}}$), 34.5 ($C_{4\text{-piperidinyl}}$), 45.9 (NH–$CH_2$), 46.0 (2C, $C_{2,6\text{-piperidinyl}}$), 60.3 ($CH_3$–**C**$H_2$–), 112.3 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 127.8 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.1 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4,8\text{-naphtyl}}$), 132.0 ($C_{9\text{-naphtyl}}$), 133.1 ($C_{2\text{-naphtyl}}$), 134.6 ($C_{10\text{-naphtyl}}$), 159.6 (2C, $C_{4,6\text{-pyrimidinyl}}$), 163.6 ($C_{2\text{-pyrimidinyl}}$), 164.6 (C(O)OEt) ppm. HRMS (ESI), calc. for $C_{23}H_{32}N_4O_4S$ 455.1753, found 455.1751.

*5.1.6.4. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylmethyl]-amino}-pyrimidine-5-sodium carboxylate (**12**).* **12** was obtained from **11** analogously as described in Section 5.1.3.

Crystallized in diethyl ether, yield = 81%. ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.10–1.28 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.38–1.55 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.67–1.85 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.21–2.38 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.05 (d, J = 6.1 Hz, 2H, C**H**$_2$–NH), 3.62–3.77 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 7.64–7.80 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 8.07 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.11–8.25 (m, 2H, $H_{4,8\text{-naphtyl}}$), 8.42 (s, 1H, $H_{1\text{-naphtyl}}$), 8.46 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 29.3 (2C, $C_{3,5\text{-piperidinyl}}$), 35.1 ($C_{4\text{-piperidinyl}}$), 46.1 (2C, $C_{2,6\text{-piperidinyl}}$), 47.3 (NH–$CH_2$), 119.8 ($C_{5\text{-pyrimidinyl}}$), 123.0 ($C_{3\text{-naphtyl}}$), 127.7 ($C_{7\text{-naphtyl}}$), 128.0 ($C_{5\text{-naphtyl}}$), 128.5 ($C_{1\text{-naphtyl}}$), 129.0 ($C_{6\text{-naphtyl}}$), 129.4 (2C, $C_{4,8\text{-naphtyl}}$), 132.0 ($C_{9\text{-naphtyl}}$), 133.3 ($C_{2\text{-naphtyl}}$), 134.5 ($C_{10\text{-naphtyl}}$), 159.1 (2C, $C_{4,6\text{-pyrimidinyl}}$), 163.1 ($C_{2\text{-pyrimidinyl}}$), 168.4 (COONa) ppm. HRMS (ESI), calc. for $C_{21}H_{22}N_4O_4S$ 427.1440, found 427.1432.

*5.1.6.5. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylmethyl]-amino}-pyrimidine-5-carboxylic acid (tetrahydropyran-2-yloxy)-amide (**13**).* **13** was obtained from **12** analogously as described in Section 5.1.4.

Yield = 90%. M.p. = 220 °C. ^{1}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.12–1.28 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.47–1.60 (m, 4H, $H_{\text{tetrahydropyranyl}}$), 1.62–1.78 (m, 5H), 2.21–2.33 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.16 (t, J = 6.0 Hz, 2H, C**H**$_2$–NH), 3.45–3.55 (m, 1H, $H_{6\text{-tetrahydropyranyl}}$), 3.66–3.75 (m, 2H, $H_{2',6\text{-piperidinyl}}$), 3.95–4.06 (m, 1H, $H_{6\text{-tetrahydropyranyl}}$), 4.90–4.94 (m, 3H, $H_{2\text{-tetrahydropyranyl}}$), 7.65–7.78 (m, 3H, $H_{3,6,7\text{-naphtyl}}$), 7.85 (t, J = 6.0 Hz, 1H, CH_2–N**H**), 8.08 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.16 (d, J = 8.7 Hz, 1H, $H_{4\text{-naphtyl}}$), 8.21 (d, J = 7.9 Hz, 1H, $H_{8\text{-naphtyl}}$), 8.42 (s, 1H, $H_{1\text{-naphtyl}}$), 8.55 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 11.42 (s, 1H, CO–NH–O) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 18.7, 25.0, 28.2 ($C_{3',4',5\text{-tetrahydropyranyl}}$), 29.2 (2C, $C_{3,5\text{-piperidinyl}}$), 34.7 ($C_{4\text{-piperidinyl}}$), 46.0 (NH–$CH_2$), 46.2 (2C, $C_{2',6\text{-piperidinyl}}$), 61.8 ($C_{6\text{-tetrahydropyranyl}}$), 101.6 ($C_{2\text{-tetrahydropyranyl}}$), 114.5 ($C_{5\text{-pyrimidinyl}}$), 123.2 ($C_{3\text{-naphtyl}}$), 128.0 ($C_{7\text{-naphtyl}}$), 128.2 ($C_{5\text{-naphtyl}}$), 128.8 ($C_{1\text{-naphtyl}}$), 129.3 ($C_{6\text{-naphtyl}}$), 129.6 (2C, $C_{4',8\text{-naphtyl}}$), 132.2 ($C_{9\text{-naphtyl}}$), 133.3 ($C_{2\text{-naphtyl}}$), 134.7 ($C_{10\text{-naphtyl}}$), 157.7 (3C, $C_{4,6\text{-pyrimidinyl}}$, C=O), 163.3 ($C_{2\text{-pyrimidinyl}}$) ppm. HRMS (ESI), calc. for $C_{26}H_{31}N_5O_5S$ 526.2124, found 526.2103.

*5.1.6.6. 2-{[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylmethyl]-amino}-pyrimidine-5-carboxylic acid hydroxyamide (**14**).* **14** was obtained from **13** analogously as described in Section 5.1.5.

Yield = 33%. M.p. = 240 °C. ^{11}H-NMR (300 MHz, [D_6]DMSO, 27 °C): δ = 1.05–1.32 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 1.40–1.61 (m, 1H, $H_{4\text{-piperidinyl}}$), 1.67–1.80 (m, 2H, $H_{3,5\text{-piperidinyl}}$), 2.20–2.34 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 3.14 (t, J = 6.2 Hz, 2H, C**H**$_2$–NH), 3.65–3.77 (m, 2H, $H_{2,6\text{-piperidinyl}}$), 7.62–7.85 (m, 4H, CH_2–N**H**, $H_{3,6,7\text{-naphtyl}}$), 8.07 (d, J = 7.9 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.15 (d, J = 8.7 Hz, $H_{4\text{-naphtyl}}$), 8.20 (d, J = 7.9 Hz, 1H, $H_{8\text{-naphtyl}}$), 8.42 (s, 1H, $H_{1\text{-naphtyl}}$), 8.54 (s, 2H, $H_{4,6\text{-pyrimidinyl}}$), 8.66 (s, 1H, NH–O**H**), 10.97 (s, 1H, N**H**–OH) ppm. ^{13}C-NMR (75 MHz, [D_6]DMSO, 27 °C): δ = 29.0 (2C, $C_{3',5\text{-piperidinyl}}$), 34.5 ($C_{4\text{-piperidinyl}}$), 45.9 (NH–$CH_2$), 46.0 (2C, $C_{2',6\text{-piperidinyl}}$), 114.8 ($C_{5\text{-pyrimidinyl}}$), 123.1 ($C_{3\text{-naphtyl}}$), 127.8 ($C_{7\text{-naphtyl}}$), 128.1 ($C_{5\text{-naphtyl}}$), 128.7 ($C_{1\text{-naphtyl}}$), 129.1 ($C_{6\text{-naphtyl}}$), 129.5 (2C, $C_{4',8\text{-naphtyl}}$), 132.0 ($C_{9\text{-naphtyl}}$), 133.1 ($C_{2\text{-naphtyl}}$), 134.6 ($C_{10\text{-naphtyl}}$), 157.5 (2C, $C_{4',6\text{-pyrimidinyl}}$), 162.3 ($C_{2\text{-pyrimidinyl}}$), 163.1 (C=O) ppm. HRMS (ESI), calc. for $C_{21}H_{23}N_5O_4S$ 442.1549, found 442.1561.

*5.1.7. Synthesis of nicotinic acid hydroxyamides **19a** and **19b***

*5.1.7.1. 6-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-nicotinic acid ethyl ester (**16a**) and 6-[1-(naphthalene-2-sulfonyl)-azepan-4-ylamino]-nicotinic acid ethyl ester (**16b**).* A mixture of 1-(naphthalene-2-sulfonyl)-piperidin-4-yl-amine [21] (0.0036 mol), 6-chloro-3-pyridinecarboxylic acid, ethyl ester (**15**) (0.0043 mol) and sodium carbonate (0.0054 ml) in DMA (10 ml) was stirred at 130 °C for 18 h, poured out into water and extracted with DCM. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated till dryness. The residue was purified by column chromatography over silica gel (15–40 μm) (eluent: DCM/MeOH 99.5:0.5). The pure fractions were collected and the solvent was evaporated. The residue (0.68 g, 43%) was crystallized from diethyl ether. The precipitate was filtered off and dried, yielding 0.477 g (30%) of **16a**. M.p. = 215 °C. ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.26 (t, J = 7.1 Hz, 3H, $CH_{3\text{-ester}}$), 1.44–1.55 (m, 2H, $H_{\text{piperidinyl}}$), 1.81–19.5 (m, 2H, $H_{\text{piperidinyl}}$), 2.60 (t, J = 10.6 Hz, 2H, $H_{\text{piperidinyl}}$), 3.57–3.64 (m, 2H, $H_{\text{piperidinyl}}$), 3.72–3.82 (m, 1H, $H_{\text{piperidinyl}}$), 4.20 (q, J = 7.1 Hz, 2H, $CH_{2\text{-ester}}$), 6.45 (d, J = 10.6 Hz, 1H, $H_{5\text{-pyridyl}}$), 7.30 (d, J = 7.1 Hz, 1H, $NH_{\text{piperidinyl}}$), 7.70–7.80 (m, 4H, H_{naphtyl}), 8.10 (d, J = 8.1 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.19 (d, J = 8.6 Hz, 1H, H_{naphtyl}), 8.23 (d, J = 8.10 Hz, 1H, H_{naphtyl}), 8.46 (s, 1H, H_{naphtyl}), 8.48 (d, J = 2 Hz, 1H, $H_{2\text{-pyrinidinyl}}$) ppm. LRMS (ESI), calc. for $C_{23}H_{25}N_3O_4S$ 439.5, found 440.2[MH]$^+$.

Similarly prepared from 1-(naphthalene-2-sulfonyl)-azepan-4-yl-amine (**12**) was **16b**: yield = 40%. M.p. = 158 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.26 (t, J = 7.1 Hz, 3H, $CH_{3\text{-ester}}$), 1.45–1.70 (m, 3H), 1.80–1.92 (m, 2H), 1.98–2.08 (m, 1H), 3.16–3.37 (m, 3H), 3.43–3.52 (m, 1H), 3.90–4.01 (m, 1H), 4.20 (q, J = 7.1 Hz, 2H, $CH_{2\text{-ester}}$), 6.43 (d, J = 9.1 Hz, 1H, $H_{5\text{-pyrinidinyl}}$), 7.38 (d, J = 7.6 Hz, 1H, $NH_{\text{homopiperidinyl}}$), 7.65–7.83 (m, 4H), 8.07 (d, J = 8.1 Hz, 1H, $H_{5\text{-naphtyl}}$), 8.15 (d, J = 8.6 Hz, 1H, H_{naphtyl}), 8.20 (d, J = 8.1 Hz, 1H, H_{naphtyl}), 8.48 (s, 1H), 8.53 (d, J = 2 Hz, 1H) ppm. LRMS (ESI), calc. for $C_{24}H_{27}N_3O_4S$ 453.7, found 454.2[MH]$^+$.

*5.1.7.2. 6-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-nicotinic acid (**17a**) and 6-[1-(naphthalene-2-sulfonyl)-azepan-4-ylamino]-nicotinic acid (**17b**).* **17a**–**b** were prepared from **16a**–**b** as sodium salts following the procedure described in Section 5.1.2. (**17a**: yield = 82%; **17b**: yield = quant.) and were used directly in the next step.

*5.1.7.3. 6-[1-(Naphthalene-2-sulfonyl)-piperidin-4-ylamino]-*N*-(tetrahydro-pyran-2-yloxy)-nicotinamide (**18a**) and 6-[1-(naphthalene-2-sulfonyl)-azepan-4-ylamino]-*N*-(tetrahydro-pyran-2-yloxy)-nicotinamide (**18b**).* **18a**–**b** were prepared from **17a**–**b** following the procedure described in Section 5.1.3.

18a crystallized from acetonitrile/diethyl ether, yield = 77%. M.p. = 129 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.40–1.60 (m, 5H), 1.60–1.72 (m, 3H), 1.90–2.00 (m, 2H), 2.57–2.68 (m, 2H), 3.44–3.51 (m, 1H, $OCH_{2\text{-tetrahydropyranyl}}$), 3.56–3.64 (m, 2H, $NCH_{\text{piperidinyl}}$), 3.67–3.74 (m, 1H, $CH_{\text{piperidinyl}}$), 3.97–4.04 (m, 1H, $OCH_{2\text{-tetrahydropyranyl}}$), 4.90 (m, 1H, $OCHO_{\text{tetrahydropyranyl}}$), 6.40 (d, J = 9.1 Hz, 1H, $H_{5\text{-pyridinyl}}$), 7.08 (d, J = 7.1 Hz, 1H, $NH_{\text{piperidinyl}}$), 7.62–7.80 (m, 4H), 8.10 (d, J = 8.1 Hz, 1H,

$H_{naphtyl}$), 8.18 (d, J = 8.6 Hz, 1H, $H_{naphtyl}$), 8.22 (d, J = 8.1 Hz, 1H, $H_{naphtyl}$), 8.33 (d, J = 2 Hz, 1H, $H_{pyridinyl}$), 8.46 (s, 1H, $H_{naphtyl}$), 11.30 (s, 1H, N**H**O–) ppm. LRMS (ESI) calc. for $C_{26}H_{30}N_4O_5S$ 510.6, found 511 .2$[MH]^+$.

18b: yield = 42%. M.p. = 164 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.48–1.78 (m, 9H), 1.80–1.91 (m, 2H), 1.98–2.07 (m, 1H), 3.15–3.38 (m, 3H), 3.43–3.53 (m, 2H), 3.89–4.08 (m, 2H), 4.90–4.95 (m, 1H, $OCH_{tetrahydropyranyl}$), 6.40 (d, J = 9.1 Hz, 1H, $H_{5\text{-pyridinyl}}$), 7.10 (d, J = 7.1 Hz, 1H), 7.66–7.76 (m, 3H), 7.81 (d, J = 8.6 Hz, 1H, $H_{3\text{-naphtyl}}$), 8.10 (d, J = 8.1 Hz, 1H, $H_{naphtyl}$), 8.15 (d, J = 8.6 Hz, 1H, $H_{naphtyl}$), 8.18 (d, J = 8.1 Hz, 1H, $H_{naphtyl}$), 8.39 (s, 1H, $H_{1\text{-naphtyl}}$), 8.48 (s, 1H, $H_{2\text{-pyridinyl}}$), 11.30 (s, 1H) ppm. LRMS (ESI), calc. for $C_{27}H_{32}N_4O_5S$ 524.6, found 525.5$[MH^+]$.

5.1.7.4. N-*hydroxy-6-[1-(naphthalene-2-sulfonyl)-piperidin-4-ylamino]-nicotinamide (**19a**) and* N-*hydroxy-6-[1-(naphthalene-2-sulfonyl)-azepan-4-ylamino]-nicotinamide (**19b**).* **19a**–**b** were prepared from **18a**–**b** following the procedure described in Section 5.1.4.

19a (0.79 $C_2HF_3O_2$) yield = 48%. M.p. = 160 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.40–1.60 (m, 2H), 1.88–2.02 (m, 2H), 2.40–2.60 (m, 2H), 3.54–3.64 (m, 3H), 6.59–6.70 (m, 1H, $H_{5\text{-pyridinyl}}$), 7.67–7.90 (m, 5H), 8.11 (d, J = 7.8 Hz, 1H, $H_{4\text{-pyridinyl}}$), 8.14–8.30 (m, 3H), 8.47 (s, 1H, $H_{2\text{-pyridinyl}}$), 9.00 (br s, 1H), 11.00 (s, 1H) ppm. LRMS (ESI), calc. for $C_{21}H_{22}N_4O_4S$ 426.5, found 427.1$[MH^+]$.

19b yield = 18%. M.p. = 213 °C, ^{1}H-NMR (400 MHz, [D_6]DMSO, 27 °C): δ = 1.44–1.71 (m, 3H), 1.73–1.95 (m, 2H), 1.96–2.10 (m, 1H), 3.10–3.70 (m, 4H), 3.87–4.02 (m, 1H, $H_{homopiperidinyl}$), 6.40–6.55 (m, 1H, $H_{5\text{-pyridinyl}}$), 7.10–7.40 (brs, 1H, $NH_{homopiperidinyl}$), 7.60–7.90 (m, 4H), 8.07 (d, J = 8.1 Hz, 1H, $H_{naphtyl}$), 8.15 (d, J = 8.6 Hz, 1H, $H_{naphtyl}$), 8.20 (d, J = 8.1 Hz, 1H, $H_{naphtyl}$), 8.34 (s, 1H, $H_{2\text{-pyridinyl}}$), 8.48 (s, 1H, $H_{1\text{-naphtyl}}$), 8.65–9.00 (br s, 1H), 10.90 (s, 1H) ppm. LRMS (ESI), calc. for $C_{22}H_{24}N_4O_4S$ 440.5, found 441.6$[MH]^+$.

5.2. Pharmacology

5.2.1. In vitro assay for inhibition of histone deacetylase

HeLa cell nuclear extracts (from Biomol) as a source of HDAC enzyme were incubated at 60 μg/ml with 2×10^{-8} M of radiolabelled peptide substrate. As a substrate for measuring HDAC activity a synthetic peptide, i.e. the amino acids 14–21 of histone H4, was used. The substrate is biotinylated at the NH_2-terminal part with a 6-aminohexanoic acid spacer, and is protected at the COOH-terminal part by an amide group and specifically [^{3}H]acetylated at lysine 16. The substrate, biotin-(6-aminohexanoic)Gly-Ala-([^{3}H]-acetyl-Lys-Arg-His-Arg-Lys-Val-NH_2), was added in a buffer containing 25 mM Hepes, 1 M sucrose, 0.1 mg/ml BSA and 0.01% Triton X-100 at pH 7.4. After 30 min the deacetylation reaction was terminated by the addition of HCl and acetic acid (final concentration 0.035 and 3.8 mM respectively). After stopping the reaction, the free ^{3}H-acetate was extracted with ethylacetate. After mixing and centrifugation, the radioactivity in an aliquot of the upper (organic) phase was counted in a β-counter.

For each experiment, controls (containing HeLa cell nuclear extract and DMSO without compound), a blank incubation (containing DMSO but no HeLa cell nuclear extract or compound) and samples (containing compound dissolved in DMSO and HeLa cell nuclear extract) were run in parallel. In first instance, compounds were tested at a concentration of 10^{-5} M. When the compounds showed activity at 10^{-5} M, a dose-response study was carried out wherein the compounds were tested at concentrations between 10^{-5} and 10^{-12} M. In each test the blank value was substracted from both the control and the sample values. The control sample represented 100% of substrate deactylation. For each sample the radioactivity was expressed as a percentage of the mean value of the controls. IC_{50}-values (concentration of the drug, needed to reduce the amount of HDAC activity to 50% of the control) were computed using probit analysis for graded data.

5.2.2. Determination of anti-proliferative activity on A2780 cells

All compounds tested were dissolved in DMSO and further dilutions were made in culture medium. Final DMSO concentrations never exceeded 0.1% (v/v) in cell proliferation assays. Controls contained A2780 cells and DMSO without compound and blanks contained DMSO but no cells. MTT was dissolved at 5 mg/ml in PBS. A glycine buffer comprised of 0.1 M glycine and 0.1 M NaCl buffered to pH 10.5 with NaOH (1 N) was prepared (all reagents were from Merck).

The human A2780 ovarian carcinoma cells (a kind gift from Dr. T.C. Hamilton [Fox Chase Cancer Center, Pennsylvania, USA]) were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 μg/ml gentamicin and 10% fetal calf serum. Cells were routinely kept as monolayer cultures at 37 °C in a humidified 5% CO_2 atmosphere. Cells were passaged once a week using a trypsin/EDTA solution at a split ratio of 1:40. All media and supplements were obtained from Life Technologies. Cells were free of mycoplasma contamination as determined using the Gen-Probe Mycoplasma Tissue Culture kit (supplier: BioMérieux).

Cells were seeded in NUNC™ 96-well culture plates (Supplier: Life Technologies) and allowed to adhere to the plastic overnight. Densities used for plating were 1500 cells per well in a total volume of 200 μl medium. After cell adhesion to the plates, medium was changed and drugs and/or solvents were added to a final volume of 200 μl. Following 4 days of incubation, medium was replaced by 200 μl fresh medium and cell density and viability was assessed using an MTT-based assay. To each well, 25 μl MTT solution was added and the cells were further incubated for 2 h at 37 °C. The medium was then carefully aspirated and the blue MTT-formazan product was solubilized by addition of 25 μl glycine buffer followed by 100 μl of DMSO. The microtest plates were shaken for 10 min on a microplate shaker and the absorbance at

540 nm was measured using an Emax 96-well spectrophotometer (Supplier: Sopachem).

Within an experiment, the results for each experimental condition are the mean of three replicate wells. For initial screening purposes, compounds were tested at a single fixed concentration of 10^{-6} M. For active compounds, the experiments were repeated to establish full concentration-response curves. For each experiment, controls (containing no drug) and a blank incubation (containing no cells or drugs) were run in parallel. The blank value was subtracted from all control and sample values. For each sample, the mean value for cell growth (in absorbance units) was expressed as a percentage of the mean value for cell growth of the control. IC_{50}-values (concentration of the drug, needed to reduce cell growth to 50% of the control) were computed using probit analysis for graded data [23].

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