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Full Paper

Synthesis of Sulfonamides and Evaluation of Their Histone Deacetylase (HDAC) Activity

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Abstract: A simple synthesis of sulfonamides **4**–**22** as novel histone deacetylase (HDAC) inhibitors is described. The key synthetic strategies involve *N*–sulfonylation of *L*–proline benzyl ester hydrochloride (**2**) and coupling reaction of *N*–sulfonyl chloride **3** with amines in high yields. It was found that several compounds showed good cellular potency with the most potent compound **20** exhibiting an IC_{50} = 2.8 μM in vitro.

Keywords: HDAC; *N*–Sulfonylation; Coupling reaction; Anticancer.

Introduction

Histone acetylation and deacetylation play fundamental roles in the modulation of chromatin topology and the regulation of gene transcription [1]. Histone deacetylase (HDAC) inhibitors that inhibit proliferation and induce differentiation and/or apoptosis of tumor cells in culture and in animal models have been identified [2]. A number of structurally diverse histone deacetylase inhibitors have shown potent antitumor efficacy with little toxicity *in vivo* in animal models. Recently, HDAC inhibitors have emerged as an exciting new class of potential anticancer agents for the treatment of solid and hematological malignancies [3]. Current research priorities are to better characterize the

biological roles and biochemical features of HDAC inhibitors. In addition, efforts to identify optimal HDAC inhibitors for anticancer therapeutics are underway [4]. More recently, the Angibaud group [5] reported the preparation of a series of pyrimidyl-5-hydroxamic acids having significant HDAC activity in human tumor cell lines. The Kalvinsh group [6] demonstrated that a series of novel sulfonamide derivatives were synthesized and evaluated for their ability to inhibit human HDAC. The Delorme group [7] developed of potential antitumor agents as a new set of sulfonamide derivatives. The Trivedi group [8] described the QSAR modeling of sulfonamide inhibitors of HDAC.

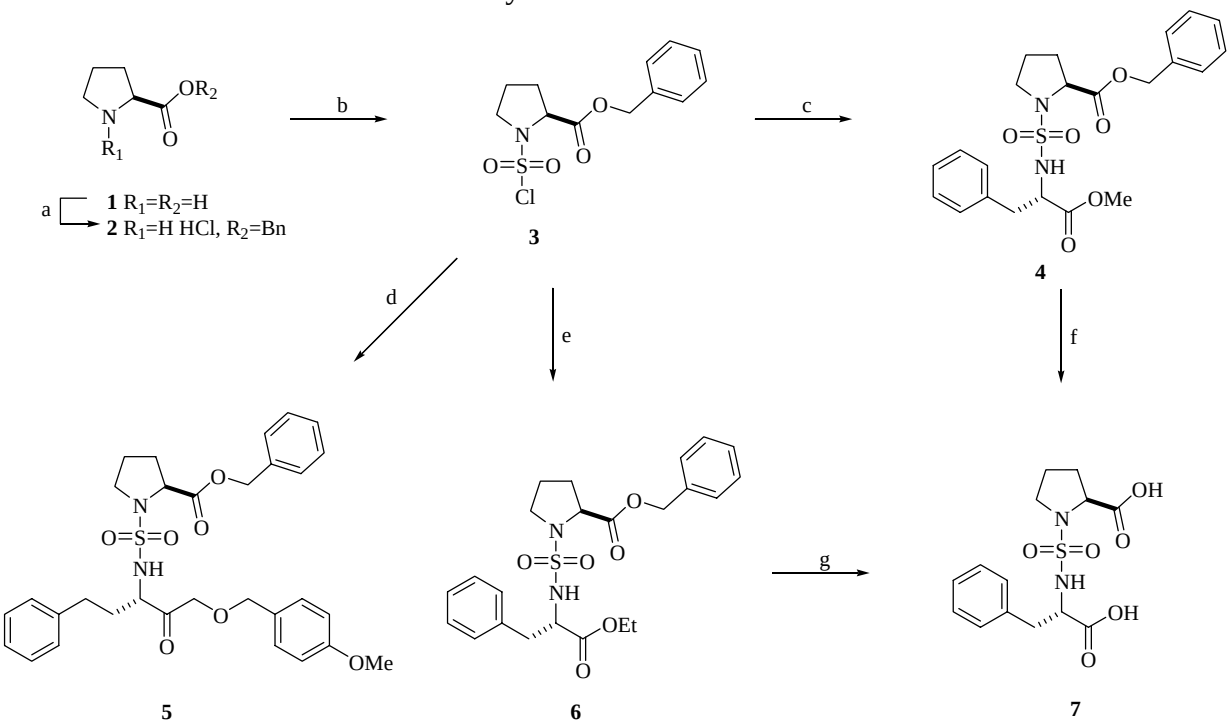
In the context of our medicinal chemistry program dealing with the development of new potent anticancer agents, we required sulfonyl chloride **3** as a key fragment in order to generate novel HDAC inhibitors. We wish to report herein an efficient synthesis in good yields of sulfonamides **4–22**, starting from *L*–proline (**1**) via benzylation, sulfonylation, and coupling reaction and the evaluation of their anti-proliferative inhibiting potency.

Results and Discussion

Chemistry

A series of sulfonamides **4–22** was prepared from (*L*)–proline (**1**) as a starting material, which was condensed with benzyl alcohol in the presence of thionyl chloride in dichloromethane to give *L*–proline benzyl ester hydrochloride (**2**) in 68% yield [9].

Scheme 1. Synthesis of sulfonamides **4–7**.

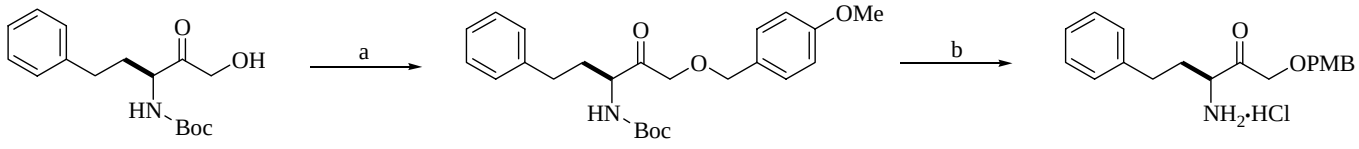


Benzyl alcohol, $SOCl_2$, CH_2Cl_2, rt, 20 h, (68%); (b) SO_2Cl_2, TEA, 4-DMAP, toluene, 0 ºC, 2 h, (72%); (c) *L*-phenylalanine methyl ester·HCl, DIPEA, 4-DMAP, CH_2Cl_2, rt, 16 h, (90%); (d) **10**, DIPEA, 4-DMAP, CH_2Cl_2, rt, 16 h, (80%); (e) *L*-phenylalanine ethyl ester·HCl, DIPEA, 4-DMAP, CH_2Cl_2, rt, 16 h, (93%); (f) LiOH, THF/H_2O, 0 ºC to rt, 4 h, (80%); (g) 1*N*-NaOH/ EtOH, rt, 16 h, (71%).

Compound **2** was treated with sulfuryl chloride (SO_2Cl_2) in toluene to generate in 72% yield the key intermediate **3** [10], which was subsequently coupled with *L*-phenylalanine methyl ester hydrochloride and *L*–phenylalanine ethyl ester hydrochloride in the presence of diisopropylethylamine (DIPEA) and 4-dimethylaminopyridine (4-DMAP) in dichloromethane to afford **4** and **6** in 90% and 93% yields, respectively.

Sulfonamides **4** and **6** were readily hydrolyzed by lithium hydroxide aqueous solution or 1*N*-sodium hydroxide aqueous solution to give acid **7** in 80% and 71% yields, respectively. In this hydrolysis reaction, basic hydrolysis (1*N*-NaOH/MeOH or LiOH, H_2O_2, THF/H_2O) is more favorable than acidic hydrolysis (3*N*-HCl/THF-H_2O, TFA/CH_2Cl_2) for preparation of acid **7** due to the higher yield and ease of handling. Furthermore, LiOH conditions afforded a superior yield for comparing with 1*N*-NaOH condition (Scheme 1).

Scheme 2. Synthesis of PMB amine **10**.



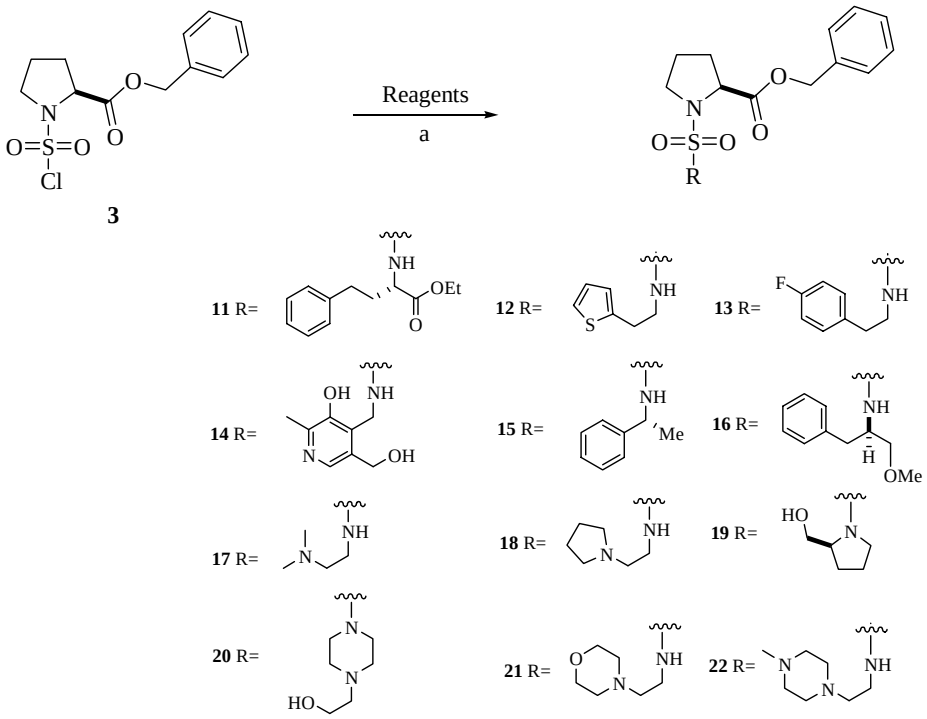
8 **9** **10**

(a) 4-Methoxybenzyl-2,2,2-trichloroacetimidate, $Sc(OTf)_3$, CH_2Cl_2, 0 ^oC to rt, 20 min, (97%); (b) 3*N*-HCl, 1,4-dioxane 0 ^oC to rt, 2 h, (64%).

To generate sulfonamide **5**, PMB-amine **10** was prepared from compound **8** [11] which was protected with freshly prepared 4-methoxybenzyl-2,2,2-trichloroacetimidate (commercially available *p*-methoxybenzyl alcohol was treated with Cl_3CCN in the presence of 1,5,7-triazabicyclo[4.4.0]dec-5-ene) and catalytic amount of scandium triflate to give **9** [12], which was subsequently treated with 3*N*-HCl aqueous solution in 1,4-dioxane in order to removal of *N*-Boc group to thus afford **10** in 62% yield (over two steps) (Scheme 2). Interestingly, the condensation reaction of sulfonyl chloride **3** with **10** was took place smoothly to generate **5** in low yield. Unfortunately, compound **5** was unstable, and isolation and characterization were problematic.

Sulfonyl chloride **3** was then coupled with several amines [amines including aromatic rings; (*S*)-(+)-ethyl-4-phenylbutyrate-2-amine, 2-thiopheneethanamine, 4-fluorophenethylamine, pyridoxamine, (*R*)-(+)-α-methylbenzylamine, and (*S*)-(+)-α-(methoxymethyl)phenethylamine; amines including aliphatic groups; *N*,*N*-dimethylethylenediamine; 1-(2-aminoethyl)pyrrolidine, (*S*)-(+)-2-(hydroxylmethyl)pyrrolidine, *N*-(2-hydroxyethyl)piperazine, *N*-(2-aminoethyl)morpholine, and 2-(4-methylpiperazin-1-yl)-ethylamine] in the presence DIPEA and 4-DMAP in dichloromethane to give sulfonamides **11**–**22** in high yields (Scheme 3).

Scheme 3. Synthesis of sulfonamides **11–22**.



(a) Reagents, DIPEA, 4-DMAP, CH_2Cl_2, rt, 3–16 h, (80%–95%).

Biological Activity

The *in vitro* growth inhibiting potency of sulfonamides **4** and **6**–**22** were evaluated and the results are summarized in Table 1. We found that potent inhibition was observed with piperazine-sulfonamide **20**, while compounds **4**, **6**, **11**–**12**, **15**, **17**–**19**, and **21** did not possess HDAC activities. When aromatic groups and pyrrolidine groups were used as coupling agents (compounds **4**, **6**, **11**–**12**, **15**, and **18**–**19**), the resulting compounds exhibited significantly reduced HDAC activity. Interestingly, piperazine–based sulfonamides **20** and **21** were showed higher *in vitro* growth inhibiting potency when compared to pyrrolidine–based sulfonamides **18** and **19**.

Table 1. HDAC and growth inhibiting potency of novel sulfonamides **4** and **6**–**22**.

Compound	IC_{50} cells (μM)[a]
7	40.3
13	36.8
14	25.3
16	21.5
20	2.8
22	12.3
4, **6**, **11**–**12**, **15**, **17**–**19**, and **21**	>100
Sodium butyrate[b]	140
Trichostatin A[c]	0.0046

[a] The values are means of three experiments.

[b,c] Reference materials.

Conclusions

In conclusion, a simple preparation of novel histone deacetylase (HDAC) inhibitors has been described. The key synthetic strategies involve *O*–benzylation, *N*–sulfonylation, and coupling reactions carried out in high yields. Compound **20** exhibited the most potent HDAC activity among these analogues. We have found that piperazine–based sulfonamides **20** and **21** showed improved growth inhibiting potency *in vitro*. In addition, the novel sulfonamides **7**, **13**, **14**, **16**, **20** and **22**) showed better HDAC activity than sodium butyrate. Although all prepared sulfonamides were exhibited less HDAC activity than trichostatin A as a compared material, we have expected that simple syntheses of new sulfonamide moieties and key fragments are useful for the modification of histone deacetylase (HDAC) inhibitors.

Experimental

General

Reactions requiring anhydrous conditions were performed with the usual precautions for rigorous exclusion of air and moisture. Tetrahydrofuran was distilled from sodium benzophenone ketyl prior to use. Thin layer chromatography (TLC) was performed on precoated silica gel G and GP uniplates from Analtech and visualized with 254–nm UV light. Flash chromatography was carried out on silica gel 60 [Scientific Adsorbents Incorporated (SAI), particle size 32–63 μm, pore size 60 Å]. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker DPX 500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Infrared (IR) spectra were obtained on an ATI Mattson FT/IR spectrometer. Mass spectra were recorded with a Waters Micromass ZQ LC–Mass system and high resolution mass spectra (HRMS) were measured with a Bruker BioApex FTMS system by direct injection using an electrospray interface (ESI). Elemental analyses were performed on a CE instruments Model 1110 elemental analyzer. When necessary, chemicals were purified according to the reported procedures [13].

Biology: In Vitro Inhibition of Histone Deacetylase

Histone deacetylase fraction was prepared as described by Yoshida *et al*. [14]. Human leukemia K562 (2.5 x 10^{8}) cells were disrupted in buffer-A (15 mM potassium phosphate buffer, pH 7.5, containing 5% glycerol and 0.2 mM EDTA, 15 mL). The nuclei were collected by centrifugation (35000*g*, 10 min) and resuspended with buffer-A (15 mL) containing 1 M $(NH_4)_2SO_4$. After sonication, the supernatant was collected by centrifugation, and ammonium sulfate was added to make the final concentration 3.5 M. After stirring for 1 h at 0 °C, the precipitate was collected by centrifugation, dissolved with buffer-A (4 mL), and dialyzed against buffer-A (2000 mL). The dialysate was loaded onto a mono Q HR 5/5 column (Pharmacia) equilibrated with buffer-A and eluted with a linear gradient of 0–1 M-NaCl in buffer-A (30 mL). A single peak of histone deacetylase activity was eluted around 0.4 M-NaCl, and the fraction was stored at –80 °C until use. Inhibition of

histone deacetylase was estimated as described by Yoshida *et al*. with slight modifications [14]. 3H-Labeled histone was prepared by the method of Yoshida *et al*.: 3 K562 cells (108 cells) were incubated in growth medium (25 mL) containing 0.5 mCi/mL [3H]sodium acetate (152.8 GBq/mmol; NEN) and 5 mM sodium butyrate at 37 °C [14]. Histone deacetylase inhibitory activity of test compound was measured as follows: the mixture (total volume 50 µL) containing the above histone deacetylase fraction (2 µL), 3H labeled histone (100 µg/mL), and test compound (5 µL) was incubated for 10 min at 37 °C. [3H]Acetic acid, which was liberated from 3H-labeled histone, was extracted with ethyl acetate, and radioactivity was measured by a liquid scintillation counter.

Chlorosulfonyl-L-proline benzyl ester (**3**)

To a stirred solution of *L*-proline benzyl ester hydrochloride (**2**, 2.4 g, 10.0 mmol) in dry toluene (25 mL) were added dropwise TEA (2.3 g, 21.0 mmol) and DMAP (0.12 g), followed by addition of sulfuryl dichloride (2.7 g, 20.0 mmol) in dry toluene (15 mL) at –10 °C and the mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with dichloromethane (70 mL) and washed with sat'd. aqueous NH_4Cl solution (80 mL) and water (100 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 15% ethyl acetate in hexanes) to give **3** (1.45 g, 48%) as a beige solid. R_f = 0.4 (15% ethyl acetate in hexanes); $[\alpha]^{24}_D$ –105.0 (c 0.6, $CHCl_3$); mp 65 °C (lit. [15] mp
65–66 °C); IR (neat, NaCl) 3474, 3412, 1638, 1618, 1457, 1124, 837 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.46–7.32 (m, 5H), 5.24 (s, 2H), 4.47 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.79–3.73 (m, 1H), 3.63–3.57 (m, 1H), 2.34–2.31 (m, 1H), 2.21–2.15 (m, 1H), 2.13–2.06 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 169.4, 135.0, 128.6, 128.5, 128.1, 67.8, 62.7, 51.7, 31.0, 24.8; HRMS calcd. for $C_{12}H_{14}NO_4SClNa$ 326.0230 $[M+Na]^+$, found 326.0240.

General procedure for coupling reaction of chlorosulfonyl-L-proline benzyl ester (**3**) *and several amines*

To a solution of chlorosulfonyl-*L*-proline benzyl ester (**3**, 0.3 g, 1.0 mmol) in dichloromethane (10 mL) were added DIPEA (0.19 g, 1.5 mmol) and DMAP (0.12 g, 0.1 mmol), followed by addition of amines (1.1 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with dichloromethane (10 mL) and washed with sat'd aqueous NH_4Cl solution (10 mL) and brine (10 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, ethyl acetate-hexane-methanol; 15:80: 5, *v/v*) to afford sulfonyl-*L*-proline benzyl esters **4**–**22**.

N-(L-Phenylalanine methyl ester)sulfonyl-L-proline benzyl ester (**4**). Yield: 90%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –12.5 (c 0.40, $CHCl_3$); IR (neat, NaCl) 3474, 3412, 1638, 1618, 1457, 1124, 837 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.42–7.18 (m, 10H), 5.20 (s, 2H), 4.56 (brs, 1H), 4.38 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 1H), 3.25 (dd, *J* = 7.0, 7.0 Hz, 1H), 3.13

(dd, *J* = 5.5, 5.5 Hz, 1H), 3.00–2.87 (m, 2H), 2.20–2.14 (m, 1H), 2.00–1.93 (m, 1H), 1.85–1.80 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 172.2, 172.0, 137.1, 135.7, 135.3, 129.5, 129.2, 128.5, 128.4, 128.3, 128.1, 126.7, 67.3, 61.0, 57.0, 52.7, 48.3, 39.4, 31.2, 25.1; HRMS calcd. for $C_{22}H_{27}N_2O_6S$: 447.1590 $[M+H]^+$, found: 447.1609; Anal. calcd. C 59.27, H 5.76, N 6.38; found: C 59.18, H 5.87, N 6.27.

N-(L-Phenylalanine ethyl ester)sulfonyl-L-proline benzyl ester (**6**). Yield: 93%; viscous oil; R_f = 0.4 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v*/*v*); $[\alpha]^{25}_D$ –105.2 (*c* 1.5, $CHCl_3$); IR (neat, NaCl) 3288, 3064, 3029, 2981, 1740, 1604, 1455, 1340, 1151, 1020, 859 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.41–7.18 (m, 10H), 5.41 (brs, 1H), 5.21 (dd, *J* = 8.0, 8.0 Hz, 2H), 4.47 (dd, *J* = 4.0, 4.0 Hz, 1H), 4.30–4.23 (m, 1H), 4.21 (q, *J* = 4.5 Hz, 2H), 3.52–3.41 (m, 2H), 2.84–2.65 (m, 2H), 2.32–2.23 (m, 1H), 2.22–2.12 (m, 1H), 2.08–1.90 (m, 4H), 1.32 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.3, 172.1, 140.6, 135.3, 128.5, 128.4, 128.3, 128.1, 126.1, 67.3, 61.9, 61.4, 56.0, 48.6, 35.2, 31.7, 31.2, 25.2, 14.6; HRMS calcd. for $C_{24}H_{30}N_2O_6SNa$: 497.1722 $[M+Na]^+$, found: 497.1743.

N-[(S)-Ethly 4-phenylbutanoate)sulfamoyl]-L-proline benzyl ester (**11**). Yield: 87%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v*/*v*); $[\alpha]^{25}_D$ –105.2 (*c* 0.5, $CHCl_3$); IR (neat, NaCl) 3288, 3064, 3029, 2981, 1740, 1604, 1455, 1340, 1151, 1020, 859 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.41–7.18 (m, 10H), 5.41 (brs, 1H), 5.21 (dd, *J* = 8.0, 8.0 Hz, 2H), 4.47 (dd, *J* = 4.0, 4.0 Hz, 1H), 4.30–4.23 (m, 1H), 4.21 (q, *J* = 4.5 Hz, 2H), 3.52–3.41 (m, 2H), 2.84–2.65 (m, 2H), 2.32–2.23 (m, 1H), 2.22–2.12 (m, 1H), 2.08–1.90 (m, 4H), 1.32 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.3, 172.1, 140.6, 135.3, 128.5, 128.4, 128.3, 128.1, 126.1, 67.3, 61.9, 61.4, 56.0, 48.6, 35.2, 31.7, 31.2, 25.2, 14.6; HRMS calcd. for $C_{24}H_{30}N_2O_6SNa$: 497.1722 $[M+Na]^+$, found: 497.1743.

1-[2-Thiophenethylsulfamoyl)]-L-proline benzyl ester (**12**). Yield: 90%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v*/*v*); $[\alpha]^{25}_D$ –34.8 (*c* 0.5, $CHCl_3$); IR (neat, NaCl) 3302, 3067, 3034, 2954, 2881, 1743, 1638, 1455, 1330, 1148, 1017, 849 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.52–7.30 (m, 5H), 7.24–7.14 (m, 1H), 7.00–6.83 (m, 2H), 5.29–5.11 (m, 2H), 4.75–4.60 (m, 1H), 4.52–4.42 (m, 1H), 3.53–3.28 (m, 4H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.36–2.24 (m, 1H), 2.12–1.90 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.5, 140.5, 135.3, 128.6, 128.4, 128.3, 127.0, 125.6, 124.0, 67.3, 61.3, 48.7, 44.7, 31.3, 30.6, 25.2; HRMS calcd. for $C_{18}H_{22}N_2O_4S_2Na$: 417.0919 $[M+Na]^+$, found: 417.0927; Anal. calcd. C 54.80, H 5.62, N 7.10; found: C 54.66, H 5.88, N 6.96.

1-[2-(4-Fluorophenethylsulfamoyl)]-L-proline benzyl ester (**13**). Yield: 85%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v*/*v*); $[\alpha]^{25}_D$ –31.2 (c 0.25, $CHCl_3$); IR (neat, NaCl) 3297, 3067, 3036, 2955, 2881, 1747, 1602, 1510, 1455, 1328, 1219, 1149, 1076, 834 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.41–7.32 (m, 5H), 7.20–7.12 (m, 2H), 7.03–6.95 (m, 2H), 5.29 (dd, *J* = 12.0, 12.0 Hz, 2H), 4.62 (t, *J* = 6.0 Hz, 1H), 4.45 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.46–3.38 (m, 1H), 3.37–3.25 (m, 3H), 2.80 (t, *J* = 7.0 Hz, 2H), 2.32–2.21 (m, 1H), 2.07–2.00 (m, 1H), 1.98–1.90 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 172.5, 162.4, 160.5, 135.3, 134.0, 130.2, 128.5, 128.3, 128.1, 115.4, 115.3, 67.2, 61.2, 48.7, 44.6, 35.5, 31.3, 25.2; HRMS calcd. for $C_{20}H_{23}N_2O_4SFNa$: 429.1260 $[M+Na]^+$, found: 429.1272; Anal. calcd. C 59.10, H 5.70, N 6.89; found: C 59.21, H 5.83, N 6.72.

1-[(R)-Methylbenzylsulfamoyl]-L-proline benzyl ester (**15**). Yield: 93%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –17.5 (*c* 0.4, $CHCl_3$); IR (neat, NaCl) 3288, 3032, 2977, 2879, 1747, 1605, 1497, 1332, 1151, 1084, 873 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.25 (m, 10H), 5.20 (dd, *J* = 12.0, 12.0 Hz, 2H), 5.14–5.05 (m, 1H), 4.73–4.65 (m, 1H), 4.40 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.31–3.19 (m, 2H), 2.16–2.07 (m, 1H), 2.01–1.94 (m, 1H), 1.92–1.76 (m, 2H), 1.53 (s, 3/2H), 1.51 (s, 3/2H); ^{13}C-NMR ($CDCl_3$) δ 172.5, 143.4, 135.4, 128.5, 128.3, 128.1, 127.4, 126.1, 67.2, 60.9, 53.6, 48.7, 31.3, 25.0, 24.2; HRMS calcd. for $C_{20}H_{24}N_2O_4SNa$: 411.1354 $[M+Na]^+$, found: 411.1367.

1-[(1S)-Methoxymethyl-2-phenethylsulfamoyl]-L-proline benzyl ester (**16**). Yield: 89%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –67.3 (c 0.30, $CHCl_3$); IR (neat, NaCl) 3288, 3030, 2980, 2892, 1749, 1603, 1498, 1331, 1149, 1083, 854 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.41–7.21 (m, 10H), 5.20 (dd, *J* = 12.0, 12.0 Hz, 2H), 4.76 (d, *J* = 9.0 Hz, 1H), 4.40 (dd, *J* = 4.5, 4.5 Hz, 1H), 3.90–3.81 (m, 1H),), 3.44 (dd, *J* = 4.5, 4.5 Hz, 1H), 3.37 (s, 3H), 3.31 (dd, *J* = 3.0, 3.0 Hz, 1H), 3.22 (dd, *J* = 8.5, 8.5 Hz, 1H), 2.96–2.84 (m, 3H), 2.24–2.16 (m, 1H), 2.01–1.94 (m, 1H), 1.86–1.79 (m, 2H); ^{13}C-NMR ($CDCl_3$) δ 172.4, 137.9, 135.5, 129.6, 128.5, 128.3, 128.2, 128.1, 126.4, 73.2, 67.1, 61.0, 59.2, 54.9, 48.3, 38.7, 31.3, 25.3; HRMS calcd. for $C_{22}H_{28}N_2O_5SNa$: 455.1617 $[M+Na]^+$, found: 455.1623; Anal. calcd. C 61.09, H 6.52, N 6.48; found: C 60.95, H 6.41, N 6.37.

1-[4-(S)-2-Hydroxymethyl)pyrrolidine-1-sulfonyl]-L-proline benzyl ester (**19**). Yield: 80%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –62.3 (*c* 0.6, $CHCl_3$); IR (neat, NaCl) 3519, 3064, 3034, 2957, 2881, 1746, 1655, 1456, 1337, 1147, 1017, 826 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.41–7.30 (m, 5H), 5.19 (s, 1H), 5.18 (s, 1H), 4.49 (dd, *J* = 3.5, 4.0 Hz, 1H), 3.94–3.87 (m, 1H), 3.63 (dd, *J* = 4.5, 4.5 Hz, 1H), 3.60–3.39 (m, 4H), 3.31–3.22 (m, 1H), 2.73 (brs, 1H), 2.32–2.21 (m, 1H), 2.06–1.93 (m, 4H), 1.89–1.75 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.4, 135.3, 128.6, 128.5, 128.4, 128.3, 128.2, 128.0, 67.3, 65.5, 61.8, 61.2, 50.1, 49.4, 31.4, 29.3, 25.3, 25.0; HRMS calcd. for $C_{17}H_{24}N_2O_5SNa$: 391.1304 $[M+Na]^+$, found: 391.1299.

1-[4-(2-Hydroxyethyl)piperazine-1-sulfonyl]-L-proline benzyl ester (**20**). Yield: 81%; viscous oil; R_f = 0.4 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –35.7 (c 0.60, $CHCl_3$); IR (neat, NaCl) 3416, 3065, 3034, 2950, 2879, 2821, 1747, 1455, 1340, 1154, 1016, 875 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.40–7.30 (m, 5H), 5.17 (d, *J* = 4.0 Hz, 2H), 4.42 (dd, *J* = 3.5, 3.5 Hz, 1H), 3.62 (t, *J* = 5.5 Hz, 2H), 3.57–3.44 (m, 1H), 3.43–3.36 (m, 1H), 3.32–3.19 (m, 4H), 2.53 (t, *J* = 5.5 Hz, 2H), 2.50–2.41 (m, 5H), 2.30–2.20 (m, 1H), 2.04–1.92 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.4, 135.4, 128.5, 128.3, 128.0, 67.1, 67.0, 61.1, 57.0, 53.4, 48.8, 39.4, 31.4, 25.3; HRMS calcd. for $C_{18}H_{28}N_3O_5S$: 398.1750 $[M+H]^+$, found: 398.1762; Anal. calcd. C 54.39, H 6.85, N 10.57; found: C 54.17, H 6.67, N 10.38

1-[2-(4-Methylpiperazin-1-yl)ethylsulfamoyl]-L-proline benzyl ester (**22**). Yield: 88%; viscous oil; R_f = 0.3 (80:15:5 *n*-hexane-ethyl acetate-methanol, *v/v*); $[\alpha]^{25}_D$ –34.0 (c 0.60, $CHCl_3$); IR (neat, NaCl) 3286, 3064, 3034, 2956, 2817, 1745, 1455, 1330, 1148, 1012, 863 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.39–7.31 (m, 5H), 5.17 (dd, *J* = 12.5, 12.5 Hz, 2H), 4.45 (dd, *J* = 4.0, 4.0 Hz, 1H), 3.52–3.37 (m, 2H), 3.31–3.20 (m, 1H), 3.19–3.11 (m, 1H), 3.10–2.98 (m, 1H), 2.64–2.33 (m, 10H), 2.29 (s, 3H), 2.27–2.21 (m, 1H), 2.07–1.94 (m, 3H); ^{13}C-NMR ($CDCl_3$) δ 172.4, 135.4, 128.5, 128.4, 128.3, 128.0, 127.3, 126.8, 67.1,

61.0, 56.4, 55.1, 52.6, 48.8, 39.7, 31.4, 25.3; HRMS calcd. for $C_{19}H_{31}N_4O_4S$: 411.2066 $[M+H]^+$, found: 411.2073; Anal. calcd. C 55.59 H 7.37, N 13.65; found: C 55.71, H 7.58, N 13.31.

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Sample Availability: Samples of the compounds are available from authors.