Bioorganic & Medicinal Chemistry 20 (2012) 2111–2118

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Potent norovirus inhibitors based on the acyclic sulfamide scaffold

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ARTICLE INFO

Article history:
Received 11 October 2011
Revised 11 January 2012
Accepted 19 January 2012
Available online 2 February 2012

Keywords:
Norovirus
Inhibitor
Acyclic sulfamide

ABSTRACT

The development of small molecule therapeutics to combat norovirus infection is of considerable interest from a public health perspective because of the highly contagious nature of noroviruses. A series of amino acid-derived acyclic sulfamide-based norovirus inhibitors has been synthesized and evaluated using a cell-based replicon system. Several compounds were found to display potent anti-norovirus activity, low toxicity, and good aqueous solubility. These compounds are suitable for further optimization of pharmacological and ADMET properties.

1. Introduction

Noroviruses (genus *Norovirus*) are human pathogens that belong to the *Caliciviridae* family.[1] Noroviruses are highly contagious, single-stranded RNA, non-enveloped viruses that are responsible for food borne and water borne acute gastroenteritis.[2–5] More than 21 million cases of acute gastroenteritis are due to norovirus infection in the US each year.[6] Outbreaks of acute gastroenteritis commonly occur in crowded settings, such as schools, cruise ships, and hospitals. There is currently no vaccine or antiviral agent for the management of norovirus infection; thus, there is an urgent and unmet need for the development of antiviral therapeutics for norovirus infection.[1,7,8]

As part of an ongoing research program related to the discovery of agents with anti-norovirus activity, we have recently described the use of cyclosulfamide-based derivatives as potent inhibitors of noroviruses[9] and have also identified additional chemotypes that exhibit anti-norovirus activity in a cell-based replicon system using a scaffold hopping strategy.[10] Further studies, including structure–activity relationship studies, have also demonstrated that functionalized piperazine derivatives also display noteworthy activity against noroviruses.[11,12] Furthermore, we have recently used a structure-based approach to design di- and tri-peptidyl inhibitors of Norwalk virus 3C protease and have demonstrated that these compounds are potent inhibitors of the protease in vitro and also exhibit anti-norovirus activity in a cell-based system.[13] We describe herein the results of our studies related to the inhibition of noroviruses by *acyclic* sulfamide derivatives using a cell-based replicon system.

1.1. Chemistry

The syntheses of *N*-(*m*-phenoxybenzyl)-substituted acyclic sulfamide derivatives proceeded uneventfully and are shown in Schemes 1–3. Briefly, chlorosulfonyl isocyanate was added dropwise to *tert*-butanol kept in an ice bath and the resulting mixture was stirred with an appropriate amino acid hydrochloride (Gly, Ala, Nva, Met) in the presence of triethylamine, yielding compounds **1a–d**. The sulfamides were selectively alkylated with *m*-phenoxybenzyl alcohol using the Mitsunobu reaction to give compounds **2a–d** (Scheme 1). Removal of the Boc group transformed compound **2a** into compound **3a**. Hydrolysis of the methyl ester followed by deblocking gave compounds **5a–d** which were subsequently coupled to 2-(morpholino)ethylamine to give compounds **6a–d**. Compounds **6a–b** and **6d** were converted to the corresponding hydrochloride salts 7**a–b** and **6d** using 4 M HCl in dioxane (Scheme 1).

Treatment of compound **2a** with sodium hydride followed by the addition of iodomethane or benzyl bromide gave compounds **8a–b** (Scheme 2). Hydrolysis of the methyl ester followed by deblocking gave compounds **9a–b** which were then coupled with 2-(morpholino)ethylamine to give compounds **11a–b**. These were subsequently converted to their corresponding salts **12a–b** using 4 M HCl/dioxane. Coupling of acid **5a** with an array of structurally-diverse amines (Scheme 3) yielded compounds **13a–g**. Compounds **13h** and **13i** were obtained from the hydrolysis of compounds **13d** and **13e**, respectively.

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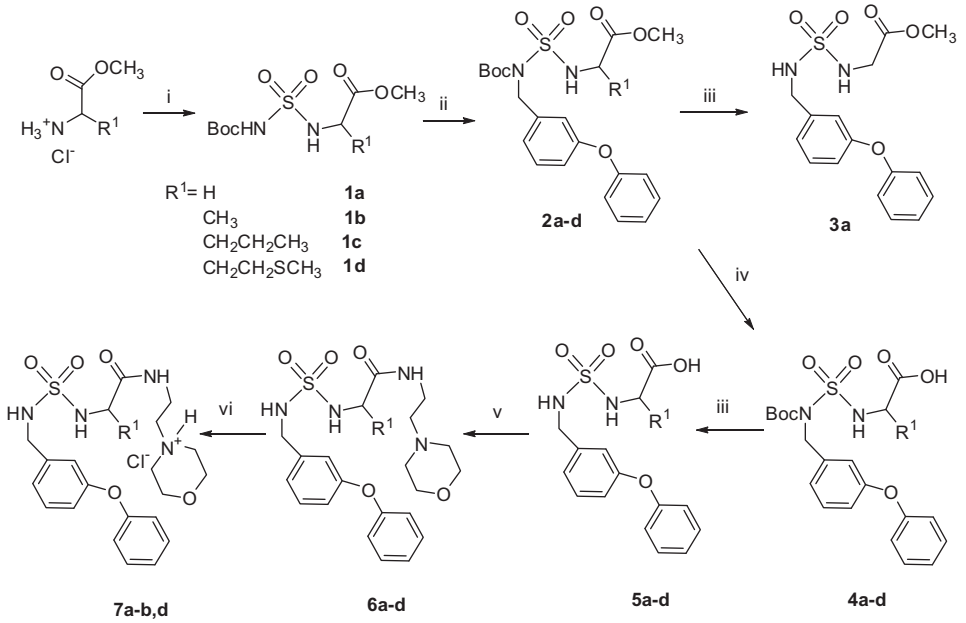
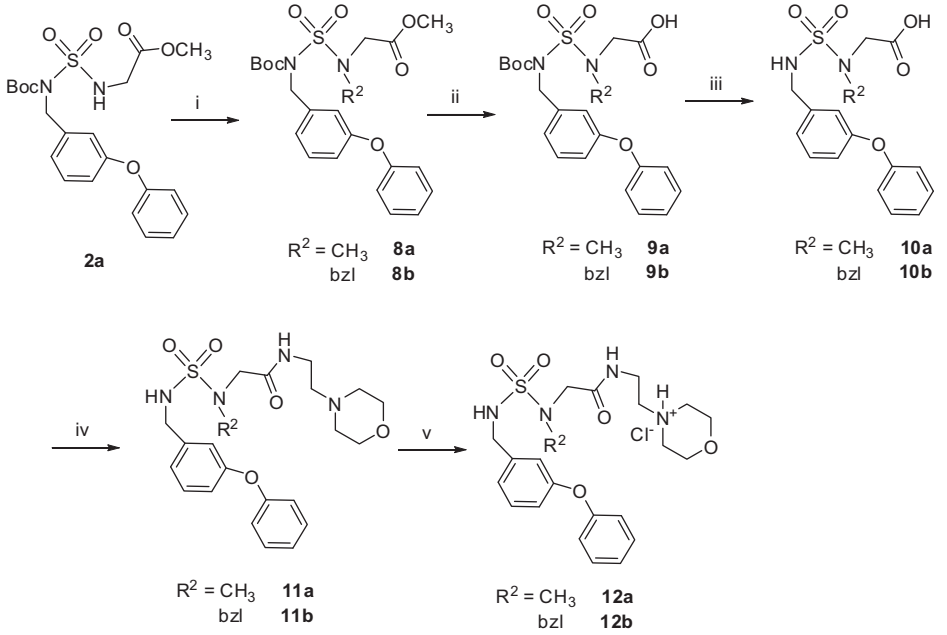
doi:10.1016/j.bmc.2012.01.030



Scheme 1. Reagents and conditions: (i) $ClSO_2NCO/t$-BuOH/CH_2Cl_2, then TEA/CH_2Cl_2/0 °C; (ii) *m*-phenoxybenzyl alcohol/Ph_3P/DEAD/THF; (iii) TFA; (iv) LiOH/aq THF; (v) CDI/THF, then 2-amino ethyl morpholine; (vi) 4 M HCl in 1,4-dioxane.



Scheme 2. Reagents and conditions: (i) NaH/CH_3CN, then R^2I; (ii) LiOH/aq THF; (iii) TFA; (iv) CDI/THF, then 2-aminoethyl morpholine; (v) 4 M HCl/1,4-dioxane.

1.2. Biochemical studies

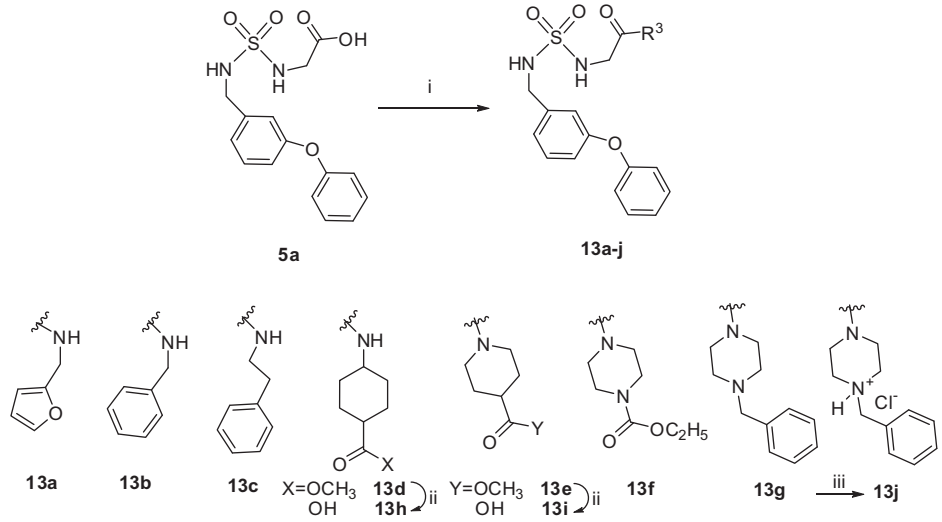
The effects of the synthesized compounds were examined in NV replicon-harboring cells (HG23 cells) and the results are summarized in Table 1. Detailed procedures for studying the antiviral effects using HG23 cells have been reported elsewhere[14–17] (see also Section 3).

2. Results and discussion

Noroviruses are highly contagious viruses that are the primary cause of viral gastroenteritis. They constitute an important health problem, as well as a potential bioterrorism threat. There are currently no therapeutics for the treatment of norovirus infection.[1,7,8]

2.1. Design rationale

The design of acyclic sulfamide norovirus inhibitors (structure (II), Fig. 1) was based on the following considerations: (a) *cyclic* sulfamide derivatives (structure (I), Fig. 1) have been shown to have low micromolar antiviral activity against noroviruses in a cell-based replicon system[9]; (b) the presence of the *m*-phenoxyphenyl moiety enhances anti-norovirus activity; (c) norovirus inhibition is sensitive to the nature of the heterocyclic ring (structure



Scheme 3. Reagents and conditions: (i) CDI/RHF, then amine; (ii) LiOH/aq dioxane; (iii) 4 M HCl.

(I), Fig. 1)[10] and, (d) the potential use of natural and unnatural amino acids provide distinct advantages in terms of stereochemical control, structural diversity, and physical properties. We reasoned that amino acid-derived *acyclic* sulfamide derivatives (structure (II), Fig. 1) may possess anti-norovirus activity by interacting with the same cellular and/or viral molecular target(s) as (I). Furthermore, the high synthetic tractability and multiple points of diversity associated with compound (II) were additional significant features that rendered (II) worthy of exploration. Thus, acyclic sulfamide derivatives **6a–d** were designed to mimic cyclosulfamide derivatives **B** and **C**[9–11] (Fig. 1), with a morpholine moiety attached for making the corresponding water-soluble hydrochloride salts. In addition, short linear alkyl chains were initially used to probe and explore the contribution of hydrophobic interactions. The potency of methionine-derived compound **6d** compared favorably to those of cyclic compounds **A–C** (Fig. 1) and had an improved therapeutic index >27 relative to compounds **A–C** which can be largely ascribed to an improvement in cellular cytotoxicity. It should be noted that the corresponding hydrochloride salt (compound **7d**, Table 1) of compound **6d** had a comparable ED_{50} and a greatly improved TD_{50} (therapeutic index ~54, Table 1). The hydrochloride salt (compound **7a**, Table 1) of glycine derivative **6a** was the least toxic and had the highest therapeutic index (TI ~65). Compounds **7a** and **7d** displayed 3- to 4-fold improvement in their therapeutic indices when compared to cyclic sulfamides **A–C** (Fig. 1). These observations prompted us to keep the glycine core constant throughout the rest of the optimization process.

Given R^1 as hydrogen, a methyl or benzyl group was introduced at R^2 before the removal of Boc and ester hydrolysis. Compound **11a**, substituted with a methyl group at R^2 gave a two-fold improvement in both ED_{50} and TD_{50} compared to its parent compound **6a**, whereas compound **11b** with R^2 = benzyl had a slightly improved potency and ~8-fold higher toxicity versus compound **11a**. The insensitivity of the ED_{50}s to substitution at R^2 (with and without substitution) suggests that the sulfamide N–H does not engage in hydrogen bonding interactions. Further insights into the nature of the interactions these compounds engage in will have to await the establishment of the identity of the molecular target. It should be noted that the acyclic sulfamides described herein do not inhibit NV 3C protease. Hydrochloride salt **12a** had a lower TI than its parent compound **11a** (TI ~24 vs ~63), while compounds **11b** and **12b** had comparable TIs.

The R^3 diversity site was probed by synthesizing an array of structurally-diverse amides (compounds **13a–j**, Scheme 3) which in some cases incorporated polar groups in their structure to enhance aqueous solubility. Among compounds **13a–j**, potency and

Table 1
Cytotoxicity and anti-norovirus activity of the synthesized compounds in replicon-harboring cells (HG23 cells)

Compound	ED_{50}[a] (μM)	TD_{50}[a] (μM)
3a	>10	ND[b]
6a	8.5 ± 1.9	165 ± 15
6b	>10	ND
6c	>10	ND
6d	4.5 ± 2.1	124 ± 25
7a	5.1 ± 1.5	330 ± 32
7b	>10	ND
7d	4.3 ± 2.1	230 ± 34
11a	5.2 ± 2.5	320 ± 24
11b	3.8 ± 2.1	45 ± 13
12a	10.1 ± 4.3	235 ± 45
12b	3.9 ± 2.8	55 ± 12
13a	>10	ND
13b	8.1 ± 2.7	47 ± 15
13c	>10	ND
13d	>10	75 ± 21
13e	>10	ND
13f	8.2 ± 3.5	130 ± 25
13g	5.5 ± 2.4	145 ± 20
13h	>10	ND
13i	>10	ND
13j	5.7 ± 2.8	120 ± 14

[a] Average of two independent experiments ± standard deviation.
[b] ND: not determined due to high ED_{50} value.

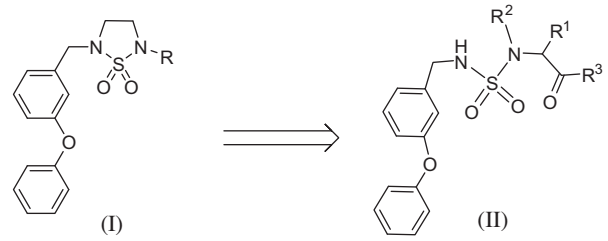


Figure 1. Design rationale of acyclic norovirus inhibitor.

cytotoxicity were sensitive to the nature of R^3. Compound **13g** and the corresponding hydrochloride salt **13j** had the best profile with TIs 26 and 21, respectively.

In summary, the studies described herein have demonstrated that functionalized acyclic sulfamides exhibit potent anti-norovirus activity in a cell-based replicon system. Taken together, several acyclic sulfamide derivatives (**6d,** 7a, **7d, 11a, and 13g**) exhibited improved therapeutic indices compared to the corresponding cyclic sulfamide derivatives. These results, coupled with the synthetic tractability and multiple points of diversity present in (II), hold significant promise for the development of anti-norovirus therapeutics. The results of ongoing studies aimed at probing further general structure (II) using natural and unnatural amino acids and each year elucidating the mechanism of action of this class of compounds, will be reported in due course.

3. Experimental section

3.1. General

The ^{1}H spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, and Bachem). Silica Gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates to determine the compound purity. The TLC plates for all the compounds were eluted using two different solvent systems and visualized using iodine and/or UV light. Each individual compound was identified as a single spot on TLC plate (purity was >95% as evidenced by ^{1}H NMR).

3.2. Synthesis of compounds 1a–d. Representative synthesis

3.2.1. Methyl 2-(*N*-(*tert*-butoxycarbonyl)sulfamoylamino) acetate 1a

To a solution of *N*-chlorosulfonyl isocyanate (11.32 g; 80 mmol) in dry methylene chloride (120 mL) cooled in an ice bath was added dropwise a solution of *t*-butyl alcohol (5.93 g; 80 mmol) in dry methylene chloride (120 mL). The resulting mixture was added dropwise to a mixture of glycine methyl ester hydrochloride (10.05 g; 80 mmol) and triethylamine (16.16 g; 160 mmol) in dry methylene chloride (120 mL) kept at 0 °C. The ice bath was removed and the reaction mixture was allowed to stir at room temperature overnight. The resulting solution was washed sequentially with 5% aqueous HCl (3 × 100 mL) and brine (100 mL). The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and the solvent was removed on the rotary evaporator, leaving compound **1a** as a white solid (14.56 g; 68% yield), mp 98–100 °C. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 3.79 (s, 3H), 3.99 (d, 2H), 5.70 (t, J = 23.1 Hz, 1H), 7.38 (s, 1H).

3.3. Compounds 1b–d were prepared using a similar procedure

3.3.1. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)sulfamoylamino) propanoate 1b

White solid, (9.69 g; 69% yield), mp 102–104 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H), 3.79 (s, 3H), 4.20–4.25 (m, 1H), 5.79 (d, 1H).

3.3.2. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)sulfamoylamino) pentanoate 1c

White solid, (11.81 g; 76% yield), mp 103–105 °C. ^{1}H NMR ($CDCl_3$): δ 0.97 (t, J = 22.7 Hz, 3H), 1.38–1.53 (m, 2H), 1.47 (s, 9H), 1.60–1.83 (m, 2H), 3.79 (s, 3H), 4.19 (m, 1H), 5.65 (d, 1H).

3.3.3. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)sulfamoylamino)-4-(methylthio)butanoate 1d

White solid, (4.44 g; 52% yield), mp 78–80 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H), 1.95–2.03 (m, 2H), 2.05 (s, 3H), 2.60 (t, J = 27.3, 2H), 3.79 (s, 3H), 5.90 (d, 1H), 7.42 (s, 1H).

3.3.4. Methyl 2-(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoylamino)acetate 2a

To a solution of triphenyl phosphine (4.52 g; 17.2 mmol) and 3-phenoxybenzyl alcohol (3.44 g; 17.2 mmol) in THF (20 mL) kept in an ice bath was added a solution of **1a** (4.61 g; 17.2 mmol) and diisopropyl azodicarboxylate (95%, 3.66 g; 17.2 mmol) in THF (20 mL). The reaction mixture was stirred at 0 °C for 2 h and monitored by TLC. The solvent was removed on the rotary evaporator, leaving a yellow oil which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to yield a white solid (6.33 g; 82% yield), mp 75–77 °C. ^{1}H NMR ($CDCl_3$): δ 1.49 (s, 9H), 3.65 (d, 2H), 3.72 (s, 3H), 4.79 (s, 2H), 4.77 (t, J = 13.6 Hz, 1H), 6.90–7.03 (m, 4H), 7.10 (d, 2H), 7.30–7.39 (m, 3H).

3.4. Compounds 2b–d were synthesized using a similar procedure

3.4.1. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl)sulfamoylamino)-propanoate 2b

White solid, (6.25 g; 70% yield), mp 41–43 °C. ^{1}H NMR ($CDCl_3$): δ 1.29 (d, 3H), 1.49 (s, 9H), 3.69 (s, 3H), 3.75–3.89 (m, 1H), 4.69–4.87 (q, J = 63.6 Hz, 2H), 5.99 (d, 1H), 6.90–7.06 (m, 4H), 7.05–7.15 (t, J = 27.3 Hz, 2H), 7.23–7.37 (m, 3H).

3.4.2. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl)sulfamoylamino)-pentanoate 2c

White solid, (3.22 g; 65% yield), mp 63–65 °C. ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 27.7 Hz, 3H), 1.23–1.79 (m, 4H), 1.49 (s, 9H), 3.69 (s, 3H), 3.75–3.89 (q, J = 27.7 Hz, 1H), 4.68–4.87 (q, J = 63.6 Hz, 2H), 5.85 (d, 1H), 6.92 (d, 1H), 7.0 (d, 2H), 7.10 (d, 2H), 5.25–5.35 (m, 5H).

3.4.3. (*RS*)-Methyl 2-(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl)sulfamoylamino)-4-(methylthio)butanoate 2d

Colorless oil, (3.41 g; 65% yield). ^{1}H NMR ($CDCl_3$): δ 1.49 (s, 9H), 2.55 (t, J = 19.0 Hz, 2H), 3.70 (s, 3H), 3.90 (q, J = 23.8 Hz, 1H), 4.68–4.88 (q, J = 76.2 Hz, 2H), 6.0 (d, 2H), 6.93 (d, 2H), 7.0 (s, 2H), 7.11 (m, 2H), 7.28–7.40 (m, 3H).

3.4.4. Methyl 2-(*N*-(3-phenoxybenzyl)sulfamoylamino)acetate 3a

Compound **2a** (3.6 g; 8.0 mmol) was treated with trifluoroacetic acid (20 mL) and stirred at room temperature for 20 min while monitoring the disappearance of the starting material by TLC. Excess trifluoroacetic acid was removed on the rotary evaporator, leaving a yellow residue which was treated with diethyl ether (10 mL). The solvent was removed and the residue was washed twice with diethyl ether, leaving a yellow oil (2.95 g; 100% yield). ^{1}H NMR ($CDCl_3$): δ 3.75 (s, 3H), 3.80 (s, 2H), 4.21 (s, 2H), 4.91–5.09 (s, 1H), 6.90–7.15 (m, 6H), 7.23 (m, 3H). HRMS (ESI) calculated m/z for $C_{16}H_{19}N_2O_5S$ $[M+H]^+$ 351.1015; found 351.1610.

3.4.5. 2-(*N*-(*tert*-Butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoylamino)acetic acid 4a

A solution of compound **2a** (18.3 g; 40.7 mmol) in THF (100 mL) was treated with a solution of lithium hydroxide (0.96 g; 40.7 mmol) in water (40 mL). The reaction mixture was stirred at room temperature for 1.5 h until the staring material disappeared (as shown by TLC). The solvent was removed and the residue was diluted with water (50 mL). The basic solution was extracted with

ethyl ether (2 × 25 mL). The pH of the aqueous layer was adjusted to ~2 with 6 M HCl (~20 mL) and the acidified solution was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried using anhydrous sodium sulfate, filtered, and concentrated, leaving a white solid (14.87 g; 85% yield), mp 115–117 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H), 3.71 (d, 2H), 4.73 (s, 2H), 6.90–7.19 (m, 5H), 7.37–7.44 (m, 4H), 8.10 (t, J = 15.8 Hz, 1H).

3.4.6. (*RS*)-2-(*N*-(*tert*-Butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoylamino)propanoic acid 4b

White solid (2.06 g; 71% yield), mp 130–132 °C. ^{1}H NMR ($CDCl_3$): δ 1.40 (d, 3H), 1.43 (s, 9H), 3.83–3.97 (m, 1H), 4.69–4.89 (q, J = 55.4 Hz, 2H), 5.97 (d, 1H), 6.95 (d, 1H), 7.0 (m, 2H), 7.05–7.15 (m, 2H), 7.28–7.38 (m, 4H).

3.4.7. (*RS*)-2-(*N*-(*tert*-Butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoylamino)pentanoic acid 4c

White solid (1.44 g; 63% yield), mp 117–119 °C. ^{1}H NMR ($CDCl_3$): δ 0.85–0.95 (t, J = 27.7 Hz, 3H), 1.30–1.50 (m, 2H), 1.42 (s, 3H), 1.53–1.80 (m, 2H), 3.81–3.88 (m, 1H), 4.68–4.89 (q, J = 64.6 Hz, 2H), 5.84 (d, 1H), 6.92 (d, 1H), 7.0 (d, 2H), 7.12 (m, 2H), 7.25–7.37 (m, 3H).

3.4.8. (*RS*)-2-(*N*-(*tert*-Butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoylamino)-4-(methylthio)butanoic acid 4d

White solid (3.11 g; 97% yield), mp 181–183 °C. ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 1.83–2.00 (m, 2H), 2.05 (s, 3H), 2.57 (t, J = 18.8 Hz, 2H), 3.98–4.02 (m, 1H), 4.64–4.89 (q, J = 70.3 Hz, 2H), 6.02 (d, 1H), 6.93 (d, 1H), 7.00 (m, 3H), 7.11 (t, J = 18.8 Hz, 2H), 7.25–7.37 (m, 3H).

3.4.9. 2-(*N*-(3-Phenoxybenzyl)sulfamoylamino)acetic acid 5a

Compound **4a** (1.50 g, 3.44 mmol) was treated with trifluoroacetic acid (15 mL) and the reaction mixture was stirred at room temperature for 30 min. Excess trifluoroacetic acid was removed, leaving a yellow residue which was treated with diethyl ether (10 mL). The solvent was removed on the rotary evaporator and the same process was repeated two more times to remove traces of acid, leaving a white solid (1.18 g; 100% yield), mp 97–99 °C. ^{1}H NMR (DMSO): δ 3.58 (s, 2H), 4.02 (s, 2H), 6.89 (d, 1H), 7.00–7.19 (m, 4H), 7.21–7.44 (m, 4H).

3.4.10. (*RS*)-2-(*N*-(3-Phenoxybenzyl)sulfamoylamino)propanoic acid 5b

White solid (1.10 g; 91% yield), mp 130–132 °C. ^{1}H NMR ($CDCl_3$): δ 1.20 (d, 3H), 3.80–3.98 (m, 1H), 4.00 (m, 2H), 6.91 (d, 1H), 7.01 (d, 2H), 7.09–7.20 (m, 2H), 7.30–7.43 (m, 5H).

3.4.11. (*RS*)-2-(*N*-(3-Phenoxybenzyl)sulfamoylamino)pentanoic acid 5c

White solid (0.84 g; 92% yield), mp 78–80 °C. ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 28.1 Hz, 3H), 1.31–1.50 (m, 2H), 1.52–1.80 (m, 2H), 3.95–4.03 (q, J = 28.1 Hz, 3H), 4.09 (s, 2H), 5.09 (d, 1H), 6.89–7.08 (m, 4H), 7.15 (t, J = 18.2 Hz, 1H), 7.26–7.39 (m, 3H).

3.4.12. (*RS*)-4-(Methylthio)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)butanoic acid 5d

Light yellow oil (1.62 g; 73% yield). ^{1}H NMR ($CDCl_3$): δ 1.79–1.95 (m, 2H), 1.21 (s, 3H), 2.43–2.60 (m, 2H), 4.03–4.20 (s, 3H), 5.80 (s, 1H), 6.85 (d, 1H), 6.93–7.12 (m, 4H), 7.21–7.37 (m, 4H).

3.4.13. *N*-(2-Morpholinoethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamide 6a

To a solution of compound **5a** (0.48 g; 1.42 mmol) in 15 mL dry THF was added carbonyldiimidazole (0.23 g; 1.42 mmol and the solution was stirred at room temperature for 20 min. A solution of 4-(2-aminoethyl)-morpholine (0.185 g; 1.42 mmol) in THF (5 mL) was added and the solution was stirred at room temperature overnight. The solvent was removed, leaving a yellow oil which was taken up in ethyl acetate (40 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, leaving a crude product which was purified by flash chromatography (silica gel/ethyl acetate/hexane) to give a light yellow oil (0.24 g; 38% yield). ^{1}H NMR ($CDCl_3$): δ 2.40–2.49 (m, 6H), 3.34–40 (q, J = 22.7 Hz, 2H), 3.63 (s, 2H), 3.63–3.70 (m, 4H), 4.20 (s, 2H), 6.65 (s, 1H), 6.95 (d, 1H), 7.00 (m, 3H), 7.05–7.18 (m, 2H), 7.30–7.40 (m, 3H). HRMS (ESI) calculated m/z for $C_{21}H_{29}N_4O_5S$ $[M+H]^+$ 449.1859; found 449.1854.

3.4.14. (*RS*)-*N*-(2-Morpholinoethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)propanamide 6b

White solid (0.35 g; 34% yield), mp 113–115 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (d, 3H), 2.36–2.56 (m, 6H), 3.32 (q, J = 18.8 Hz, 2H), 3.65 (t, J = 14.1 Hz, 14H), 3.84–3.93 (m, 1H), 4.20 (d, 2H), 4.95 (s, 1H), 5.05 (s, 1H), 6.55 (s, 1H), 6.95 (d, 1H), 6.99–7.18 (m, 4H), 7.26–7.40 (m,4H). HRMS (ESI) calculated m/z for $C_{22}H_{31}N_4O_5S$ $[M+H]^+$ 463.2015; found 463.2014.

3.4.15. (*RS*)-*N*-(2-Morpholinoethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)pentanamide 6c

Light yellow oil (0.20 g; 25% yield). ^{1}H NMR ($CDCl_3$): δ 0.79–1.00 (t, J = 65.6 Hz, 3H), 1.20–1.42 (m, 2H), 1.49–1.72 (m, 2H), 2.20–2.49 (m, 6H), 3.23–3.39 (m, 2H), 3.53–3.71 (m, 4H), 3.70–3.80 (q, J = 28.1 Hz, 1H), 4.19 (s, 2H), 5.30–5.50 (s, 1H), 5.60–5.80 (s, 1H), 6.79 (s, 1H), 6.82–7.17 (m, 5H), 7.20–7.40 (m, 3H). HRMS (ESI) calculated m/z for $C_{24}H_{35}N_4O_5S$ $[M+H]^+$ 491.2328; found 491.2290.

3.4.16. (*RS*)-4-(Methylthio)-*N*-(2-morpholinoethyl)-2-(*N*-(3-phenoxybenzyl)sulfamoylamino)butanamide 6d

Colorless oil (0.17 g; 9% yield). ^{1}H NMR ($CDCl_3$): δ 1.95–2.03 (q, J = 28.8 Hz, 2H), 2.09 (s, 3H), 2.35–2.50 (m, 6H), 2.50–2.69 (m, 2H), 3.23–3.43 (m, 2H), 3.64 (t, J = 18.2 Hz, 4H), 4.00–4.08 (q, J = 28.1 Hz, 1H), 4.19 (d, 2H), 5.00 (t, J = 28.1 Hz, 1H), 5.51 (d, 1H), 6.62 (t, J = 18.2 Hz, 1H), 6.94 (d, 1H), 7.00 (m, 2H), 7.02–7.18 (m, 2H), 7.29–7.38 (m, 3H). HRMS (ESI) calculated m/z for $C_{24}H_{35}N_4O_5S_2$ $[M+H]^+$ 523.2049; found 523.2036.

3.4.17. *N*-(2-Morpholinoethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamide hydrochloride 7a

Compound **6a** (0.20 g; 0.45 mmol) was treated with 4 M HCl in dioxane (10 mL; 40 mmol) and the solution was stirred at room temperature for 20 min. The solvent was removed, leaving a yellow oil which was treated with diethyl ether (20 mL) and then concentrated again. The residue was treated with a mixture of methylene chloride (5 mL) and hexane (10 mL) and the solvent was removed, leaving a yellow solid (0.10 g; 46% yield), mp 140–142 °C. ^{1}H NMR (DMSO): δ 3.10–3.23 (m, 2H), 3.40–3.49 (s, 2H), 3.51–3.69 (m, 6H), 3.88–3.93 (m, 4H), 4.03 (d, 2H), 6.90 (d, 1H), 7.00 (d, 2H), 7.05–7.19 (m, 2H), 7.30–7.43 (m, 3H), 7.55 (t, J = 21.4 Hz, 1H), 8.19 (s, 1H). HRMS (ESI) calculated m/z for $C_{21}H_{29}N_4O_5S$ $[M+H]^+$ 449.1859; found 449.1864.

3.4.18. (*RS*)-*N*-(2-Morpholinoethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)propanamide hydrochloride 7b

White solid (0.25 g; 100% yield), mp 166–168 °C. ^{1}H NMR (DMSO): δ 1.21 (d, 3H), 3.10–3.23 (m, 2H), 3.40–3.49 (q, J = 18.8 Hz, 1H), 3.51–3.69 (m, 6H), 3.88–3.93 (m, 4H), 4.03 (d, 2H), 6.90 (d, 1H), 7.00 (m, 2H), 7.07–7.19 (m, 2H), 7.30–7.43 (m, 3H), 7.55 (t, J = 26.1 Hz, 1H), 8.21 (s, 1H). HRMS (ESI) calculated m/z for $C_{22}H_{31}N_4O_5S$ $[M+H]^+$ 463.2015; found 463.2009.

3.4.19. (*RS*)-4-(Methylthio)-*N*-(2-morpholinoethyl)-2-(*N*-(3-phenoxybenzyl)sulfamoyl-amino)butanamide hydrochloride 7d

Yellow solid (0.13 g; 94% yield), mp 107–109 °C. ^{1}H NMR ($CDCl_3$): δ 1.70–1.99 (m, 2H), 2.11 (s, 3H), 2.51 (m, 2H), 2.75–2.99 (m, 2H), 3.10–3.20 (m, 1H), 3.20–3.39 (m, 2H), 3.40–3.55 (m, 1H), 3.71–4.42 (m, 7H), 5.05 (d, 2H), 6.95 (d, 1H), 7.00–7.18 (m, 3H), 7.20–7.40 (m, 5H), 7.90 (t, J = 25.0 Hz, 1H), 8.80 (s, 1H), 9.60–9.80 (s, 1H). HRMS (ESI) calculated m/z for $C_{24}H_{35}N_4O_5S_2$ $[M+H]^+$ 523.2049; found 523.2043.

3.4.20. Methyl 2-((*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoyl) (methyl)amino)acetate 8a

To a chilled solution of compound **2a** (2.25 g; 5 mmol) in acetonitrile (10 mL) was added NaH (60% w/w; 0.24 g; 6 mmol). After stirring for 30 min, iodomethane (0.85 g; 6 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred overnight. The reaction mixture was refluxed for 2.5 h and monitored by TLC. The solvent was removed, leaving a residue which was taken up in methylene chloride (75 mL) and washed with 5% HCl (3 × 25 mL), and brine (3 × 25 ml). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, leaving a yellow oil (2.05 g; 89% yield).^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 2.85 (s, 3H), 3.71 (s, 3H), 4.03 (s, 2H), 4.83 (2H), 6.93 (d, 2H), 7.0 (d, 2H), 7.12 (m, 2H), 7.29–7.38 (m, 3H).

3.4.21. Methyl 2-(benzyl(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl)sulfamoyl)amino)-acetate 8b

Colorless oil (0.36 g; 22% yield). ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H), 3.73 (s, 3H), 3.90 (s, 2H), 4.48 (s, 2H), 4.80, (s, 2H), 6.89 (d, 2H), 6.98 (d, 2H), 7.04–7.35 (m, 9H).

3.4.22. 2-((*N*-(*tert*-Butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoyl)(methyl)amino)acetic acid 9a

A solution of compound **8a** (1.87 g; 4.03 mmol) in THF (10 mL) was treated with a solution of lithium hydroxide (0.12 g; 5 mmol) in 5 mL H_2O. The reaction mixture was stirred at room temperature for 1.5 h and monitored by TLC. The solvent was removed and the residue was diluted with water (20 mL) and washed with ethyl ether (2 × 25 mL). The aqueous layer was separated and the pH was adjusted to ~2 using 6 M HCl (~3 mL). The acidified aqueous layer was extracted with ethyl acetate (2 × 40 mL) and the organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, leaving a colorless oil (1.15 g; 64% yield). ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 2.81 (s, 3H), 4.10 (s, 2H), 4.82 (s, 2H), 6.93 (d, 2H), 7.0 (d, 2H), 7.13 (d, 2H), 7.28–7.37 (m, 3H).

3.4.23. 2-(Benzyl(*N*-(*tert*-butoxycarbonyl)-*N*-(3-phenoxybenzyl) sulfamoyl)amino)acetic acid 9b

Colorless oil (0.33 g; 88% yield). ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 3.99 (s, 2H), 4.44 (s, 2H), 4.80 (s, 2H), 6.89–6.98 (m, 3H), 7.01–7.36 (m, 10H).

3.4.24. 2-(Methyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)acetic acid 10a

A solution of compound **9a** (1.0 g, 2.22 mmol) in trifluoroacetic acid (10 mL) was stirred at room temperature for 20 min while monitoring the disappearance of the starting material by TLC. Excess trifluoroacetic acid was removed, leaving a yellow residue which was treated with diethyl ether (10 mL). The solvent was removed again on the rotary evaporator and the residue was washed with ether twice, leaving a yellow oil (0.73 g; 95% yield). ^{1}H NMR ($CDCl_3$): δ 2.89 (s, 3H), 4.00 (s, 2H), 4.21 (s, 2H), 6.90–7.15 (m, 7H), 7.25–7.39 (m, 3H).

3.4.25. 2-(Benzyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)acetic acid 10b

Light yellow oil (0.20 g; 84% yield). ^{1}H NMR ($CDCl_3$): δ 3.93 (s, 2H), 4.30 (s, 2H), 4.43 (s, 2H), 4.91–4.98 (s, 1H), 6.90–7.18 (m, 5H), 7.23–7.40 (m, 8H).

3.4.26. 2-(Methyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)-*N*-(2-morpholinoethyl)acetamide 11a

To a solution of compound **10a** (0.65 g; 1.86 mmol) in dry THF (15 mL) was added carbonyldiimidazole (0.30 g; 1.86 mmol) and the solution was stirred at room temperature for 20 min (starting material disappeared, as monitored by TLC). A solution of 4-(2-aminoethyl)-morpholine (0.24 g; 1.86 mmol) in THF (5 mL) was added and the reaction mixture was stirred at room temperature overnight. The solvent was removed on the rotary evaporator, leaving a yellow oil which was taken up in ethyl acetate (40 mL), and washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried, filtered, and concentrated, leaving a light yellow oil (0.45 g; 52% yield). ^{1}H NMR ($CDCl_3$): δ 2.40–2.53 (m, 6H), 2.86 (s, 3H), 3.36–3.40 (q, J = 27.3 Hz, 2H), 3.65–3.72 (t, J = 27.3 Hz, 4H), 3.79 (s, 2H), 4.28 (d, 2H), 5.26 (t, J = 22.7, 1H), 6.71(s, 1H), 6.90–7.17 (m, 5H), 7.25–7.39 (m, 3H). HRMS (ESI) calculated m/z for $C_{22}H_{31}N_4O_5S$ $[M+H]^+$ 463.2015; found 463.1996.

3.4.27. 2-(Benzyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)-*N*-(2-morpholinoethyl)acetamide 11b

Colorless oil (0.10 g; 42% yield). ^{1}H NMR ($CDCl_3$): δ 2.42–2.53 (m, 6H), 3.25–3.35 (q, J = 23.4 Hz, 2H), 3.63–3.72 (t, J = 18.8 Hz, 4H), 3.78 (s, 2H), 4.30 (d, 2H), 4.39 (s, 2H), 5.85–5.95 (t, J = 18.8 Hz, 1H), 6.50–6.59 (s, 1 H), 6.90–7.12 (m, 6H), 7.23–7.37 (m, 7H). HRMS (ESI) calculated m/z for $C_{28}H_{35}N_4O_5S$ $[M+H]^+$ 539.2328; found 539.2336.

3.4.28. 2-(Methyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)-*N*-(2-morpholinoethyl)acetamide hydrochloride 12a

Compound **11a** (0.42 g; 0.9 mmol) was treated with 4 M HCl in dioxane (10 mL, 40 mmol) and stirred at room temperature for 20 min. Removal of the solvent left a yellow oil which was treated with diethyl ether (20 mL). The solvent was removed and the residue was treated with a mixture of methylene chloride (5 mL) and hexane (10 mL) and the solvent was removed, leaving a yellow oil (0.30 g; 67% yield). ^{1}H NMR ($CDCl_3$): δ 2.79–2.98 (s, 2H), 2.99 (s, 3H), 3.24 (s, 2H), 3.60–3.81 (m, 6H), 3.91–4.00 (m, 2H), 4.10–4.19 (m, 2H), 4.35 (d, 2H), 6.90 (d, 2H), 6.99–7.20 (m, 5H), 7.28–7.38 (m, 3H), 7.84 (t, J = 20.0 Hz, 1H), 8.66 (t, J = 20.0 Hz, 1H). HRMS (ESI) calculated m/z for $C_{22}H_{31}N_4O_5S$ $[M+H]^+$ 463.2015; found 463.2006.

3.4.29. 2-(Benzyl(*N*-(3-phenoxybenzyl)sulfamoyl)amino)-*N*-(2-morpholinoethyl)acetamide hydrochloride 12b

Light yellow oil (0.04 g; 69% yield). ^{1}H NMR ($CDCl_3$): δ 2.80–2.95 (s, 2H), 3.21–3.29 (t, J = 27.3 Hz, 2H), 3.51–3.80 (m, 6H), 3.89–4.01 (m, 2H), 4.02–4.23 (m, 2H), 4.38 (d, 2H), 4.42 (s, 2H), 6.90 (d, 1H), 7.00 (d, 2H), 7.03–7.14 (m, 4H), 7.20–7.45 (m, 6H), 8.25 (t, J = 27.3 Hz, 1H), 8.55 (t, J = 27.3 Hz, 1H). HRMS (ESI) calculated m/z for $C_{28}H_{35}N_4O_5S$ $[M+H]^+$ 539.2328; found 539.2328.

3.4.30. *N*-(Furan-2-ylmethyl)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamide 13a

To a solution of compound **5a** (0.34 g; 1.00 mmol) in 15 mL dry THF was added carbonyldiimidazole (0.16 g; 1.00 mmol) and the reaction mixture was stirred at room temperature for 20 min. A solution of 2-furfurylamine (0.097 g; 1.00 mmol) in THF (5 mL) was added and the solution was stirred at room temperature overnight. The solvent was removed, leaving a yellow oil which was taken up in ethyl acetate (30 mL) and washed with water

(2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed, leaving a light yellow oil which was treated with diethyl ether (40 mL). A white precipitate formed which was collected by suction filtration (0.23 g; 55% yield); mp 110–112 °C. ^{1}H NMR (DMSO): δ 3.50 (d, 2H), 4.02 (d, 2H), 4.28 (d, 2H), 6.23 (s, 1H), 6.39 (s, 1H), 6.90 (d, 1H), 7.00 (d, 2H), 7.09–7.19 (m, 2H), 7.23–7.45 (m, 4H), 8.29 (t, J = 20.7 Hz, 1H). HRMS (ESI) calculated m/z for $C_{20}H_{21}N_3O_5SNa$ $[M+Na]^+$ 438.1100; found 438.1095.

3.4.31. *N*-Benzyl-2-(*N*-(3-phenoxybenzyl)sulfamoylamino) acetamide 13b

White solid (0.30 g; 69% yield), mp 142–144 °C. ^{1}H NMR (DMSO): δ 3.48 (s, 2H), 4.01 (d, 2H), 4.25 (d, 2H), 6.88 (d, 1H), 6.99 (d, 2H), 7.04–7.16 (m, 2H), 7.20–7.41 (m, 9H), 7.46 (s, 1H), 8.35 (t, J = 20.7 Hz, 1H). HRMS (ESI) calculated m/z for $C_{22}H_{23}N_3O_4SNa$ $[M+Na]^+$ 448.1307; found 448.1311.

3.4.32. *N*-Phenethyl-2-(*N*-(3-phenoxybenzyl)sulfamoylamino)acetamide 13c

White solid (0.28 g; 64% yield), mp 127–129 °C. ^{1}H NMR (DMSO): δ 2.69 (t, J = 25.0 Hz, 2H), 3.22–3.37 (m, 2H), 3.40 (d, 2H), 4.00 (d, 2H), 6.85 (d, 1H), 7.00 (d, 2H), 7.05–7.44 (m, 11H), 7.89 (t, J = 10.0 Hz, 1H). HRMS (ESI) calculated m/z for $C_{23}H_{25}N_3O_4SNa$ $[M+Na]^+$ 462.1463; found 462.1447.

3.4.33. (*RS*,*RS*)-Methyl 4-(2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamido)-cyclohexanecarboxylate 13d

Colorless oil (0.07 g; 15% yield). ^{1}H NMR ($CDCl_3$): δ 1.48–1.60 (m, 2H), 1.62–1.75 (m, 3H), 1.80–1.95 (m, 3H), 2.43–2.53 (m, 1H), 3.60 (d, 2H), 3.65 (s, 3H), 3.87–3.97 (m, 1H), 4.20 (d, 2H), 5.02–5.15 (m, 2H), 6.23 (d, 2H), 6.88–7.14 (m, 7H), 7.24–7.36 (m, 2H). HRMS (ESI) calculated m/z for $C_{23}H_{29}N_3O_6SNa$ $[M+Na]^+$ 498.1675; found 498.1676.

3.4.34. (*RS*)-Methyl 1-(2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetyl)piperidine-4-carboxylate 13e

To a solution of compound **5a** (0.34 g; 1.00 mmol) in dry THF (10 mL) was added carbonyldiimidazole (0.16 g; 1.00 mmol) and the solution was stirred at room temperature for 20 min. A solution of methyl piperidine-4-carboxylate (0.14 g; 1.00 mmol) in THF (5 mL) was added and the reaction mixture was stirred at room temperature overnight. The solvent was removed and the residue was taken up in ethyl acetate (30 mL) and washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed, leaving a light yellow oil. Addition of diethyl ether (40 mL) yielded a white precipitate which was collected by suction filtration (0.21 g; 46% yield), mp 93–95 °C. ^{1}H NMR ($CDCl_3$): δ 1.58–1.75 (m, 2H), 1.90–2.00 (m, 2H), 2.50–2.62 (m, 1H), 2.85–2.96 (t, J = 37.5 Hz, 1H), 3.00–3.11 (t, J 37.5 Hz, 1H), 3.51–3.61 (d, 1H), 3.69 (s, 3H), 3.80 (s, 2H), 4.20 (d, 2H), 4.21–4.31 (d, 2H), 4.68 (t, J = 18.8 Hz, 1H), 5.43 (s, 1H), 6.90–7.15 (m, 6H), 7.22–7.35 (m, 3H). HRMS (ESI) calculated m/z for $C_{22}H_{27}N_3O_6SNa$ $[M+Na]^+$ 484.1518; found 484.1506.

3.4.35. Ethyl 4-(2-(*N*-(3-phenoxybenzyl)sulfamoylamino) acetyl)piperazine-1-carboxylate 13f

White solid (0.38 g; 53% yield), mp 95–97 °C. ^{1}H NMR ($CDCl_3$): δ 1.23–1.32 (t, J = 27.7 Hz, 3H), 3.31 (m, 2H), 3.43–3.54 (m, 4H), 3.58 (m, 2H), 3.80 (s, 2H), 4.15–4.23 (m, 3H), 4.55 (t, J = 18.5 Hz, 1H), 5.38 (s, 1H), 6.90–7.15 (m, 7H), 7.27–7.38 (m, 2H). HRMS (ESI) calculated m/z for $C_{22}H_{28}N_4O_6SNa$ $[M+Na]^+$ 499.1627; found 499.1614.

3.4.36. *N*-(4-Benzylpiperazine)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamide 13g

White solid (0.39 g; 53% yield), mp 125–127 °C. ^{1}H NMR ($CDCl_3$): δ 2.37–2.45 (t, J = 34.6 Hz, 4H), 3.25–4.33 (t, J = 34.6 Hz, 2H), 3.53 (s, 2H), 3.55–3.43 (t, J = 34.6 Hz, 2H), 3.80 (s, 2H), 4.20 (s, 2H), 4.29–4.40 (s, 1H), 5.35–5.46 (s, 1H), 6.90–7.15 (m, 5H), 7.22–7.39 (m, 8H). HRMS (ESI) calculated m/z for $C_{26}H_{31}N_4O_4S$ $[M+H]^+$ 495.2066; found 495.2073.

3.4.37. (*RS*,*RS*)-4-(2-(*N*-(3-Phenoxybenzyl)sulfamoylamino) acetamido)cyclohexane-carboxylic acid 13h

A solution of compound **13d** (0.17 g; 0.36 mmol) in THF (5 mL) was treated with a solution of lithium hydroxide (0.03 g; 1.00 mmol) in water (1 mL) and the reaction mixture was stirred at room temperature for 0.5 h (monitored by TLC until the disappearance of the staring material). The solvent was removed on the rotary evaporator and the residue was diluted with water (20 mL). The pH was adjusted to ~2 using 6 M HCl (~2 mL) and the aqueous layer was extracted with ethyl acetate (2 × 30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, leaving a white solid (0.16 g; 100% yield), mp 156–158 °C. ^{1}H NMR (DMSO): δ 1.41–1.59 (m, 2H), 1.62–1.75 (m, 3H), 1.80–1.96 (m, 3H), 2.43–2.53 (m, 1H), 3.60 (d, 2H), 3.62–3.71 (m, 1H), 4.05 (d, 2H), 5.90 (d, 1H), 6.91 (d, 1H), 7.00 (d, 2H), 7.06–7.19 (m, 2H), 7.30–7.43 (m, 3H), 7.70 (d, 1H). HRMS (ESI) calculated m/z for $C_{22}H_{27}N_3O_6SNa$ $[M+Na]^+$ 484.1518; found 484.1501.

3.4.38. (*RS*)-1-(2-(*N*-(3-Phenoxybenzyl)sulfamoylamino) acetyl)piperidine-4-carboxylic acid 13i

A solution of compound **13e** (0.10 g; 0.22 mmol) in THF (5 mL) was treated with a solution of lithium hydroxide (0.03 g; 1.00 mmol) in water (1 mL) and the reaction mixture was stirred at room temperature for 0.5 h (monitored by TLC until the disappearance of the staring material). The solvent was removed on the rotary evaporator and the residue was diluted with water (10 mL). The pH was adjusted to ~2 using 6 M HCl (~2 mL) and the aqueous layer was extracted with ethyl acetate (2 × 30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated, leaving a white solid (0.06 g; 61% yield), mp 151–153 °C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.60 (m, 4H), 1.78–1.86 (m, 2H), 2.59 (m, 1H), 2.70–2.80 (t, J = 37.5 Hz, 1H), 3.00–3.10 (t, J = 37.5 Hz, 1H), 3.61–3.70 (d, 1H), 3.73 (d, 2H), 4.04 (d, 2H), 4.06–4.15 (d, 1H), 6.80 (t, J = 18.8 Hz, 1H), 6.89 (d, 1H), 7.00 (d, 2H), 7.09–7.17 (m, 2H), 7.30–7.42 (m, 3H). HRMS (ESI) calculated m/z for $C_{21}H_{25}N_3O_6SNa$ $[M+Na]^+$ 470.1362; found 470.1373.

3.4.39. *N*-(4-Benzylpiperazine)-2-(*N*-(3-phenoxybenzyl) sulfamoylamino)acetamide hydrochloride 13j

Compound **13g** (0.20 g; 0.45 mmol) was dissolved with 4 M HCl in dioxane (10 mL; 40 mmol) and stirred at room temperature for 20 min. Upon the reaction completion, the excess HCl and solvent were removed under vacuum, leaving the crude product as a yellow oil. This oil was redissolved in diethyl ether (2 × 10 mL) and then concentrated to dryness to give the pure product as a white solid (0.18 g, 63% yield), mp 168–170 °C. ^{1}H NMR (DMSO): δ 2.37–2.45 (t, J = 34.6 Hz, 4H), 3.25–4.33 (m, 2H), 3.40 (s, 2H), 3.53 (s, 2H), 3.55–3.43 (m, 2H), 4.20 (d, 2H), 4.29–4.43 (s, 1H), 6.85 (d, 1H), 7.00 (m, 2H), 7.04–7.15 (q, 29.5 Hz, 1H), 7.29–7.40 (m, 3H), 7.41–7.58 (m, 2H). HRMS (ESI) calculated m/z for $C_{26}H_{31}N_4O_4S$ $[M+H]^+$ 495.2066; found 495.2068.

3.5. Biochemical studies

One-day old, 80–90% confluent HG23 cells were treated with varying concentrations of each compound to examine its effects

on the replication of NV (0 [mock-DMSO]–10 μM) and cell cytotoxic effects (0–320 μM). At 24 or 48 h of treatment, the NV protein or genome were analyzed with Western blot analysis or qRT-PCR, respectively. The ED_{50}s of the compounds for NV genome levels were determined at 24 h post-treatment. The cytotoxic effects of the compounds on HG23 cells were determined using a cell cytotoxicity assay kit (Promega, Madison, WI) to calculate the median toxic dose (TD_{50}) at 48 h of treatment.

Acknowledgment

The generous financial support of this work by the National Institutes of Health (AI081891) is gratefully acknowledged.

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