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Design and Synthesis of Inhibitors of Noroviruses by Scaffold Hopping

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Abstract

A scaffold hopping strategy was employed to identify new chemotypes that inhibit noroviruses. The replacement of the cyclosulfamide scaffold by an array of heterocyclic scaffolds lead to the identification of additional series of compounds that possessed anti-norovirus activity in a cell-based replicon system.

Introduction

Noroviruses are single-stranded RNA, non-enveloped viruses that belong to the *Norovirus* genus of the *Caliciviridae* family. They are the cause of ~21 million cases of acute gastroenteritis in the U.S.[1] Noroviruses are highly contagious, consequently, outbreaks of acute gastroenteritis are common, particularly in schools, nursing homes, restaurants, hospitals, and cruise ships. Currently, there is no vaccine or low molecular weight antiviral drug available for the treatment of norovirus infections.

Scaffold hopping (also termed chemotype switching)[2–3] is an integral component of the drug discovery process and is an effective strategy for exploring chemical space and the discovery of back-up series of compounds. This is a powerful approach for identifying new chemotypes which display improved pharmacological and ADME/Tox characteristics, as well as address intellectual property issues. We have recently described the discovery of a new class of anti-norovirus agents that embody in their structure the cyclosulfamide scaffold (Figure 1). We describe herein the results of our studies related to the application of scaffold hopping in the discovery of new series of compounds that inhibit noroviruses by modifying the cyclosulfamide core structure (Figure 1).

Chemistry

Compounds ***2, 4–5*** and ***9a–b*** were synthesized as shown in Scheme 1. These compounds were readily synthesized by reductive amination of m-(phenoxy)benzaldehyde with excess ethylenediamine in the presence of sodium borohydride in methanol[4–5] to yield the corresponding N-substituted ethylene diamine ***1.*** Stirring overnight with carbonyldiimidazole in dioxane yielded N-substituted imidazolidinone ***2***. Alkylation of ***2*** with sodium hydride followed by methyl or t-butyl bromoacetate gave low yields of the corresponding products, consequently an alternative method involving refluxing N-(m-

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phenoxy)benzylethylene diamine ***1*** and t-butyl bromoacetate in DMF was used.[6] Compound ***3*** was cyclized to the corresponding N-substituted imidazolidinone with carbonyldiimidazole. Teatment with TFA followed by esterification and lithium borohydride reduction of the resulting ester gave alcohol ***7***. Formation of mesylate ***8*** followed by refluxing with morpholine in 95% ethanol in the presence of sodium bicarbonate yielded ***9a***. Refluxing ***8*** with piperazine gave the corresponding dimer ***9b***. Compounds ***11a–b*** were made by refluxing (m-phenoxy)benzaldehyde and 1,3-diaminopropane to yield intermediate ***10***, which was converted to compounds ***11a*** and ***11b*** by refluxing with sulfamide in pyridine or stirring with carbonyldiimidazole, respectively (Scheme 2).

Triazole derivatives ***14a–e*** were readily synthesized using click chemistry[7–8] as illustrated in Scheme 3. Tetrazole derivatives ***16a-b*** were prepared by heating nitrile ***15*** with sodium azide in DMF (Scheme 3). Imidazole derivative ***17*** was readily synthesized by reacting (m-phenoxy)benzyl alcohol with carbonyldiimidazole in acetonitrile[9] (Scheme 3).

Compound ***21*** was synthesized as illustrated in Scheme 4 using similar procedures as those described previously.[10–12]

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.[13–15]

Results and Discussion

Noroviruses constitute a significant public health problem. There are currently no drugs on the market for the treatment of norovirus infection and, furthermore, only a limited number of studies have been reported in the literature related to the development of norovirus therapeutics.[16–18] Using a cell-based replicon system, we have recently demonstrated that cyclosulfamide-based derivatives are potent inhibitors of noroviruses (Figure 1, structure (I)). Furthermore, structure-activity relationship studies indicated that anti-norovirus activity was greatly influenced by multiple factors, including the nature of the groups attached to the cyclosulfamide scaffold and the nature of the rings in the diphenyl ether moiety. Based on these findings, we have used the cyclosulfamide scaffold as the starting point of a scaffold hopping strategy aimed at identifying new chemotypes that possess enhanced binding affinity and aqueous solubility, as well as other drug-like characteristics.[19]

A conservative change involving the replacement of the SO_2 moiety in cyclosulfamide by C=O to yield a 2-imidazolidinone ring was initially made (compound ***2***). The potency of compound ***2*** was lower than that of the corresponding cyclosulfamide compound, however, there was a small improvement in the TD_{50} (Table 1). The 2-imidazolidinone scaffold was also embellished with an acidic (compound ***5)*** or basic (compound ***9a***) component to increase aqueous solubility. These compounds were either inactive (compound ***5)*** or exhibited reduced anti-norovirus activity (compound ***9a***). In contrast, dimer ***9b*** was an order of magnitude more potent than the initial cyclosulfamide hit, however, it had high toxicity (Table 1). Increasing the ring size of the cyclosulfamide or 2-imidazolidinone rings yielded compounds that were devoid of anti-norovirus activity (compounds ***11a*** and ***11b***, Table 1).

We envisaged that the replacement of the cyclosulfamide ring with a series of structurally-diverse electron-rich rings may yield compounds exhibiting greater affinity with the putative receptor. Thus, the cyclosulfamide scaffold was sequentially replaced by a triazole, tetrazole, imidazole or 1, 2, 5-thiadiazolidin-3-one 1,1 dioxide ring. A few of the triazole derivatives (compounds ***14b***, ***14d*** and ***14e***, Table 1) exhibited anti-norovirus activity,

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however, their TD_{50} values were too low. In both the 2-imidazolidinone and triazole series (compounds ***5*** and ***14c,*** Table 1), the presence of a carboxyl group was inimical to anti-norovirus activity. The tetrazole derivatives were inactive (compounds ***16a*** and ***16b,*** Table 1), while the corresponding imidazole compound was moderately active with a low therapeutic index. Replacement of the cyclosulfamide ring with the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold yielded an inactive compound (compound ***21,*** (Table 1). Taken together, these observations suggest that that the nature of the heterocyclic ring has a profound effect on the anti-norovirus activity and cytotoxicity of these compounds.

In conclusion, a scaffold hopping strategy was employed to identify new chemotypes that inhibit noroviruses. These preliminary studies suggest that pharmacological activity and cytotoxicity are impacted by subtle changes in structure. Identification of the molecular target(s) these compounds interact with should greatly facilitate exploitation of these observations and may lead to the emergence of effective anti-norovirus therapeutics.

Experimental Section

General

The ^1H 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 greater than 95%).

Representative synthesis

1-(3-Phenoxybenzyl)imidazolidin-2-one (2)—To a solution of ethylenediamine (6.00 g; 100 mmol) in 65 mL methanol kept in an ice bath was added 3-(phenoxy)benzaldehyde (4.95 g; 25 mmol) in small portions. After the addition, sodium borohydride (0.94 g; 25 mmol) was slowly added portionwise at 0 °C. The reaction was allowed to warm to room temperature overnight with stirring. The solvent was removed and the residue was taken up in ethyl acetate (100 mL). The organic layer was washed with water (40 mL) and dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed *in vacuo* to give pure compound ***1*** as colorless oil (6.00 g; 99% yield). ^1H NMR ($CDCl_3$): δ 1.40 (s, 3H), 2.65- 2.90 (m, 4H), 3.78 (s, 2H), 6.98 (t, J = 10.1 Hz, 3H), 7.10 (t, J = 9.8 Hz, 1H), 7.20–7.40 (m, 5H). To solution of compound ***1*** (0.52 g; 2 mmol) in dry 1,4-dioxane (12 mL) was added a solution of N,N′-carbonyldiimidazole (0.40 g; 2.48 mmol) in 2 mL dry 1,4-dioxane. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed and the residue was taken up in ethyl acetate (20 mL). The organic layer was washed with 5% HCl (3 × 10 mL), brine (10 mL) and then dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was evaporated to give a white solid ***2*** (0.32 g; 56% yield), mp 108–109 °C. ^1H NMR ($CDCl_3$): δ 3.28–3.43 (m, 4H), 4.37 (s, 2H), 5.54 (s, 1H), 6.89–7.37 (m, 9H). HRMS (ESI) calculated m/z for $C_{16}H_{16}N_2O_2Na$ $[M+Na]^+$ 291.1109; found 291.1084.

tert-Butyl 2-(2-(3-phenoxybenzylamino)ethylamino)acetate (3)—To a solution of t-butyl 2-bromoacetate (9.84 g; 66.6 mmol) in dry DMF (90 mL) kept in an ice bath was added dropwise a solution of N-(m-phenoxy)benzyl ethylenediamine (48.00 g; 200 mmol) in dry DMF (600 mL) over 1.5 h. After the addition, the reaction was allowed to warm to room

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temperature and stirred for 16 h. DMF was removed under vacuum and the residue was taken up in ethyl acetate (500 mL) and water (400 mL). The organic layer was separated and the aqueous solution was extracted with an additional 200 mL of ethyl acetate. The combined organic extracts were dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed on the rotary evaporator. The crude product was purified using flash chromatography (silica gel/ethyl acetate/hexanes) to give compound ***3*** as a yellow oil (25.40 g; 98% yield). ^{1}H NMR ($CDCl_3$): δ 1.43 (s, 9H), 2.74 (s, 4H), 3.39 (s, 2H), 3.80 (s, 2H), 6.85–7.40 (m, 9H).

tert-Butyl 2-(2-oxo-3-(3-phenoxybenzyl)imidazolidin-1-yl)acetate (4)—Compound ***4*** was prepared using the same procedure as that used in the synthesis of compound ***2***. Yellow oil (76% yield). ^{1}H NMR ($CDCl_3$): δ 1.44 (s, 9H), 3.20–3.28 (m, 2H), 3.39–3.47 (m, 2H), 3.90 (s, 2H), 4.38 (s, 2H), 6.85–7.36 (m, 9H). HRMS (ESI) calculated m/z for $C_{22}H_{27}N_2O_4$ $[M+H]^+$ 383.1971; found 383.1968.

2-(2-Oxo-3-(3-phenoxybenzyl)imidazolidin-1-yl)acetic acid (5)—Compound ***4*** (19.00 g; 49.7 mmol) was treated with trifluoroacetic acid (150 mL) and stirred for 1 h. TFA was removed and the pH of the residue was adjusted to 10 using cold 1N NaOH. The aqueous solution was extracted with ethyl acetate (2 × 100 mL) to remove unreacted starting material and the pH of the aqueous layer was adjusted to ~1 with 6 N HCl. The solution was extracted with ethyl acetate (2 × 100 mL) and the combined organic layers were dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed to give pure compound ***5*** as a yellow oil (15.68 g; 97% yield). ^{1}H NMR ($CDCl_3$): δ 3.31–3.36 (m, 2H), 3.48–3.53 (m, 2H), 4.05 (s, 2H), 4.38 (s, 2H), 6.88–7.37 (m, 9H), 9.90 (s, 1H). HRMS (ESI) calculated m/z for $C_{18}H_{19}N_2O_4$ $[M+H]^+$ 327.1345; found 327.1359.

Methyl 2-(2-oxo-3-(3-phenoxybenzyl)imidazolidin-1-yl)acetate (6)—To 10 mL dry methanol kept in an ice bath was added dropwise thionyl chloride (1.31 g; 11 mmol), followed by the addition of compound ***5*** (3.59 g; 11 mmol) in small portions. After the addition, the reaction mixture was warmed to 40 °C for 2 h. The solvent was removed under vacuum and the residue was dissolved in 20 mL ethyl acetate. The solvent was again removed under vacuum to give compound ***6*** as a colorless oil (3.40 g; 100% yield). ^{1}H NMR ($CDCl_3$): δ 3.23–3.32 (m, 2H), 3.40–3.48 (m, 2H), 3.75 (s, 3H), 4.02 (s, 2H), 4.39 (s, 2H), 6.85–7.36 (m, 9H).

1-(2-Hydroxyethyl)-3-(3-phenoxybenzyl)imidazolidin-2-one (7)—To a solution of compound ***6*** (1.70 g; 5 mmol) in 8 mL dry THF was added dropwise a solution of 2 M $LiBH_4$ (2.5 mL; 5 mmol), followed by dropwise addition of absolute ethanol (15 mL). The reaction mixture was stirred at room temperature overnight. The reaction mixture was cooled in an ice bath and acidified with 5% aqueous HCl to pH 4. The solvent was removed under vacuum and the residue was taken up in ethyl acetate (85 mL) and washed with brine (25 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under vacuum to give compound ***7*** as a colorless oil (1.25 g; 80% yield). ^{1}H NMR ($CDCl_3$): δ 3.20–3.42 (m, 7H), 3.77 (t, J = 5.1 Hz, 2H), 4.35 (s, 2H), 6.85–7.36 (m, 9H).

2-(2-Oxo-3-(3-phenoxybenzyl)imidazolidin-1-yl)ethyl methanesulfonate (8)—To a solution of compound ***7*** (1.25 g; 4 mmol) and triethylamine (0.41 g; 4 mmol) in 10 ml dry methylene chloride was added methanesulfonyl chloride (0.50 g; 4.3 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Methylene chloride (10 mL) was added to the reaction mixture and the resulting solution was washed with saturated sodium bicarbonate (2 × 20 mL). The organic layer was separated and dried

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over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed to give compound ***8*** as a colorless oil (1.56 g; 100% yield). ^{1}H NMR ($CDCl_3$): δ 3.02 (s, 3H), 3.25 (t, J = 7.6 Hz, 2H), 3.45 (t, J = 7.6 Hz, 2H), 3.58 (t, J = 4.8 Hz, 2H), 4.35–4.40 (m, 4H), 6.88–7.37 (m, 9H).

1-(2-Morpholinoethyl)-3-(3-phenoxybenzyl)imidazolidin-2-one (9a)—A mixture of compound ***8*** (0.86 g; 2.2 mmol), morpholine (0.19 g; 2.2 mmol) and $NaHCO_3$ (1.0 g; 12 mmol) in 10 ml 95% ethanol was refluxed overnight. The solvent was removed and the residue was taken up in ethyl acetate (30 mL) and water (30 mL). The organic layer was separated, washed with 30 mL brine and then dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed to give pure compound ***9a*** as a white solid (0.65 g; 78% yield), mp 66–68 °C. ^{1}H NMR ($CDCl_3$): δ 2.43–2.54 (m, 6H), 3.17–3.23 (m, 2H), 3.32–3.41 (m, 4H), 3.70 (t, J = 4.9 Hz, 4H), 4.36 (s, 2H), 6.86–7.37 (m, 9H). HRMS (ESI) calculated m/z $C_{22}H_{28}N_3O_3$ $[M+H]^+$ 382.2131; found 382.2151.

3,3'-(2,2'-(Piperazine-1,4-diyl)bis(ethane-2,1-diyl))bis(1-(3-phenoxybenzyl)imidazo-lidin-2-one) (9b)—Compound ***9b*** was prepared using a similar procedure as that used for making compound ***9a*** using piperazine. Colorless oil (55% yield). ^{1}H NMR ($CDCl_3$): δ 2.40–2.60 (m, 12H), 3.12–3.21 (m, 4H), 3.28–3.43 (m, 8H), 4.34 (s, 4H), 6.82–7.37 (m, 18H). HRMS (ESI) calculated m/z $C_{40}H_{47}N_6O_4$ $[M+H]^+$ 675.3659; found 675.3645.

N^1-(3-Phenoxybenzyl)propane-1,3-diamine (10)—To a solution of 1, 3-diaminopropane (7.4 g; 100 mmol) in 65 mL methanol kept in an ice bath was added 3-(phenoxy)benzaldehyde (4.95 g; 25 mmol) in small portions. After the addition, sodium borohydride (0.94 g; 25 mmol) was added slowly in small portions at 0 °C. The reaction was allowed to warm to room temperature overnight with stirring. The solvent was removed and the residue was taken up in ethyl acetate (50 mL), and water (40 mL) was added. The two layers were separated and the organic layer was washed with water (40 mL) and dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed to give pure compound ***10*** as a colorless oil (6.40 g; 100% yield). ^{1}H NMR ($CDCl_3$): δ 1.38 (s, 3H), 1.64 (t, J = 7.5 Hz, 2H), 2.66 (t, J = 7.0 Hz, 2H), 2.74 (t, J = 7.0 Hz, 2H), 3.78 (s, 2H), 6.82–7.35 (m, 9H).

2-(3-Phenoxybenzyl)-1,2,6-thiadiazinane 1,1-dioxide (11a)—To a refluxing solution of sulfamide (0.48 g; 5 mmol) in anhydrous pyridine (12 mL) was slowly added compound ***10*** (1.28 g; 5 mmol) over 1h. The resulting reaction mixture was refluxed for an additional 16 h. Pyridine was removed under vacuum, and the residue was taken up in ethyl acetate (20 mL). The organic layer was washed with 5% HCl (3 × 10 mL), brine (10 mL) and then dried over anhydrous sodium sulfate. The drying agent was filtered off and the solvent was removed, leaving a crude product which was purified using flash chromatography (silica gel/ethyl acetate/hexanes) to give pure compound ***11a*** as a white solid (1.45 g; 91% yield), mp 94–96 °C. ^{1}H NMR ($CDCl_3$): δ 1.60–1.71 (m, 2H), 3.20 (t, J = 5.6 Hz, 2H), 3.49 (q, J = 6.4 Hz, 2H), 4.19 (s, 2H), 4.57 (t, J = 7.1 Hz, 1H), 6.88–7.38 (m, 9H). HRMS (ESI) calculated m/z for $C_{16}H_{19}N_2O_3S$ $[M+H]^+$ 319.1116; found 319.1129.

1-(3-Phenoxybenzyl)tetrahydropyrimidin-2(1H)-one (11b)—To solution of compound ***10*** (0.52 g; 2 mmol) in dry 1, 4-dioxane (12 mL) was added a solution of N,N′-carbonyldiimidazole (0.40 g; 2.48 mmol) in 2 mL dry 1,4-dioxane. The reaction mixture was stirred at room temperature for 18 h. The solvent was removed and the residue was taken up in ethyl acetate (20 mL). The organic layer was washed with 5% HCl (3 × 10 mL), brine (10 mL) and dried over anhydrous sodium sulfate. The drying agent was filtered off and the

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solvent was removed to give a solid. The crude product was washed with 20 mL diethyl ether to give pure compound ***11b*** as a white solid (0.32 g; 57% yield), mp 91–93 °C. ^{1}H NMR ($CDCl_3$): δ 1.80–1.92 (m, 2H), 3.19 (t, J = 5.1 Hz, 2H), 3.30 (t, J = 4.8 Hz, 2H), 4.53 (s, 2H), 6.80–7.40 (m, 9H). HRMS (ESI) calculated m/z for $C_{17}H_{19}N_2O_2$ $[M+H]^+$ 283.1447; found 283.1429.

1-(Azidomethyl)-3-phenoxybenzene (13)—A solution of m-(phenoxy)toluene (3.68 g; 20 mmol) in 45 mL CCl_4 was treated with N-bromosuccinimide (5.34 g; 30 mmol) and azo-bis(isobutyronitrile) (15 mg) and the reaction mixture was refluxed for 3 h. The solution was allowed to cool to room temperature and then placed in an ice bath. A white precipitate formed which was filtered off and the filtrate was evaporated, leaving pure compound ***1*** as a yellow oil (4.5 g; 86% yield). ^{1}H NMR ($CDCl_3$): δ 4.4 (s, 2H), 6.9–7.41 (m, 9H). This was used in the next step. To sodium azide (3.25 g; 50 mmol) in dry DMSO (80 mL) was added compound ***12*** (4.5 g; 17.1 mmol) and the reaction mixture was stirred overnight at room temperature. The reaction mixture was cooled in an ice bath and quenched with water (50 mL). The aqueous layer was extracted with ethyl ether (3 × 30 mL). The combined ethyl ether extracts were washed with water (2 × 20 mL) and dried over anhydrous sodium sulfate. The solvent was removed, leaving a crude product which was purified using flash chromatography (silica gel/methylene chloride/ hexanes) to give compound ***13*** as a colorless oil (3.31 g; 60% yield). ^{1}H NMR ($CDCl_3$): δ 4.25 (s, 2H), 6.07–7.45 (m, 9H).

2-(1-(3-Phenoxybenzyl)-1H-1,2,3-triazol-4-yl)propan-2-ol (14a)—To compound ***13*** (2.02 g; 9 mmol) was added 2-methyl-3-butyn-2-ol (0.76 g; 9 mmol), t-butyl alcohol (20 mL), water (20 mL), sodium ascorbate (0.3 g; 1.5 mmol), and $CuSO_4.5H_2O$ (60 mg). The reaction mixture was stirred overnight at ~ 50°C. Cold water (40 mL) was added, whereupon a solid formed. The solid was collected by filtration and purified using flash chromatography (silica gel/ethyl acetate/hexanes) to give compound ***14a*** as light yellow colored oil (0.40 g; 15% yield). ^{1}H NMR ($CDCl_3$): δ 1.60 (s, 6H), 2.40 (s, 1H), 5.46 (s, 2H), 6.90–7.05 (m, 4H), 7.10–7.40 (m, 6H). HRMS (ESI) calculated m/z for $C_{18}H_{20}N_3O_2$ $[M+H]^+$ 310.1556; found 310.1551.

Compounds ***14b-c*** was prepared using a similar procedure as that described above.

4-Butyl-1-(3-phenoxybenzyl)-1H-1,2,3-triazole (14b)—yellow oil (26% yield). ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 10.5Hz, 3H), 1.30–1.42 (m, 2H), 1.60–1.70 (m, 2H), 2.70 (t, J = 10.5Hz, 2H), 5.43 (s, 2H), 6.90–7.05 (m, 4H), 7.10–7.40 (m, 6H). HRMS (ESI) calculated m/z for $C_{19}H_{22}N_3O$ $[M+H]^+$ 308.1763; found 308.1742.

4-(1-(3-Phenoxybenzyl)-1H-1,2,3-triazol-4-yl)butanoic acid (14c)—white solid (21% yield). mp 75–77°C. ^{1}H NMR ($CDCl_3$): δ 1.98–2.09 (p, J = 13.3Hz, 2H), 2.50 (t, J = 6.6Hz, 2H), 2.80 (t, J = 6.6Hz, 2H), 5.42 (s, 2H), 6.98–7.40 (m, 10H), 12.02 (s, 1H). HRMS (ESI) calculated m/z for $C_{19}H_{20}N_3O_3$ $[M+H]^+$ 338.1505; found 338.1479.

1-(3-Phenoxybenzyl)-4-(trimethylsilyl)-1H-1,2,3-triazole (14d)—A solution of compound ***2*** (2.25 g; 10 mmol) in 10 mL dry DMSO was treated with trimethylsilyl acetylene (0.98 g; 10 mmol), and CuI (191 mg), and the reaction mixture was heated to 90 °C for 3.5 h. Ethyl acetate (75 mL) was added and the solution was washed with saturated ammonium chloride (100 mL) containing concentrated ammonium hydroxide (2 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried over anhydrous sodium sulfate. The mixture was filtered and the solvent was removed, leaving a crude product as a brown oil which was purified by flash chromatography (silica gel/ethyl acetate/hexanes) to give compound ***14d*** as a light yellow oil (0.70g ; 21% yield). ^{1}H NMR ($CDCl_3$): δ 0.30 (d, J = 10.7Hz, 9H), 5.50 (s,

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2H), 6.85 (d, J = 10.7Hz, 1H), 6.90–7.40 (m, 8H), 7.84 (s, 1H). HRMS (ESI) calculated m/z for $C_{18}H_{22}N_3OSi$ $[M+H]^+$ 324.1532; found 324.1510.

1-(3-Phenoxybenzyl)-1H-1,2,3-triazole (14e)—Compound ***14d*** (0.48 g; 1.5 mmol) in dry THF (6 mL) was treated with tetra n-butylammonium fluoride (0.39 g; 1.5 mmol) and the reaction mixture was stirred overnight at room temperature. The solvent was removed and the residue was partitioned between saturated ammonium chloride (8 mL) and ethyl acetate (40 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were dried using anhydrous sodium sulfate. The solvent was removed to yield compound ***14e*** as a yellow oil (0.31 g; 83% yield). ^{1}H NMR ($CDCl_3$): δ 5.50 (s, 2H), 6.85 (d, J = 10.7Hz, 1H), 6.90–7.40 (m, 8H), 7.50 (s, 1H), 7.84 (s, 1H). HRMS (ESI) calculated m/z for $C_{15}H_{13}N_3ONa$ $[M+Na]^+$ 274.0956; found 274.0942.

2-(3-Phenoxyphenyl)acetonitrile (15)—A solution of compound ***12*** (5.26 g; 20 mmol) in dry DMSO (5 mL) was added dropwise to a rapidly stirred mixture of sodium cyanide (1.06; 21.6 mmol) in 10 mL DMSO and the reaction mixture was stirred for 5 h at room temperature. Water (50 mL) was added and the solution was extracted with ethyl acetate (3 × 85 mL). The combined organic layers were washed with 30 mL brine and dried over anhydrous sodium sulfate. The solution was filtered and the solvent was evaporated to yield a light yellow oil which was purified by flash chromatography to give compound ***15*** as a colorless oil (0.90 g; 22% yield). ^{1}H NMR (DMSO-d_6): δ 4.31 (s, 2H), 6.82–7.08 (m, 5H), 7.15 (t, J = 10.7Hz, 1H), 7.30–7.41 (m, 4H).

5-(3-Phenoxybenzyl)-2H-tetrazole (16a)—To a stirred solution of compound ***15*** (2.1 g; 10 mmol) in dry DMF (12 mL) was added ammonium chloride (0.54 g; 10 mmol) and sodium azide (0.64 g; 10 mmol). The suspension was stirred for 8h at 100°C. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated to leave a crude product which was treated with water (5 mL) and the solution acidified with 5% HCl. The solution was extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with 30 mL brine and dried over anhydrous sodium sulfate. The solvent was removed to yield a crude product which was purified by flash chromatography to give compound ***16a*** as a light yellow oil (0.70 g; 28% yield). ^{1}H NMR (DMSO-d_6): δ 4.31 (s, 2H), 6.82–7.08 (m, 5H), 7.15 (t, J = 10.7Hz, 1H), 7.30–7.41 (m, 4H). HRMS (ESI) calculated m/z for $C_{14}H_{13}N_4O$ $[M+H]^+$ 253.1089; found 253.1064.

2-Methyl-5-(3-phenoxybenzyl)-2H-tetrazole (16b)—A solution of compound ***16a*** (0.37 g; 1.5 mmol) and triethylamine (0.17 g; 1.7 mmol) in dry acetonitrile (7 mL) was treated with methyl iodide (0.21 g; 1.5 mmol) with stirring. The reaction mixture was stirred at room temperature for 5 h and refluxed for 20 h. The solution was filtered and the solvent was removed to give compound ***16b*** as a colorless viscous oil (0.34 g; 87% yield). ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 3H), 4.31 (s, 2H), 6.80–7.20 (m, 6H), 7.21–7.40 (m, 3H). HRMS (ESI) calculated m/z for $C_{15}H_{15}N_4O$ $[M+H]^+$ 267.1246; found 267.1230.

1-(3-Phenoxybenzyl)-1H-imidazole (17)—3-(Phenoxy)benzyl alcohol (2 g; 10 mmol) and N,N’carbonyldiimidazole (2.42 g; 14 mmol) were dissolved in THF (10 mL) and acetonitrile (14 mL). The reaction mixture was refluxed for 7h. The solvent was removed and the residue was taken up in ethyl acetate (80 mL) and washed with water (30 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed to yield a crude product which was purified by flash chromatography (silica gel/ ethyl acetate/ hexanes) to give compound ***17*** as a colorless oil (0.6 g; 24% yield). ^{1}H NMR

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($CDCl_3$): δ 5.12 (s, 2H), 6.80–7.01 (m, 5H), 7.05–7.20 (m, 2H), 7.26–7.40 (m, 4H), 7.55 (s, 1H). HRMS (ESI) calculated m/z for $C_{16}H_{15}N_2O$ $[M+H]^+$ 251.1184; found 251.1169.

Methyl 2-(3-phenoxybenzylamino)acetate (18)—A mixture of glycine methyl ester hydrochloride (5.03 g; 40 mmol) in 25 mL dry methanol was treated with triethylamine (4.05 g; 40 mmol) and the reaction mixture was stirred for 10 minutes. A solution of benzaldehyde (4.24 g; 40 mmol) in dry methanol (12.5 mL) was added dropwise and the reaction mixture was stirred for 4 h at 0 °C under a nitrogen atmosphere. Sodium borohydride (3.03 g; 80 mmol) was added and the reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*, leaving a white solid which was washed with ether (3 × 10 mL) to give compound ***18*** (14.36 g; 100% yield). ^1H NMR ($CDCl_3$): δ 3.76 (s, 3H), 3.98 (s, 2H), 4.20 (s, 2H), 7.00–7.42 (m, 9H), 10.00 (s, 1H).

Methyl 2-((N-(tert-butoxycarbonyl)sulfamoyl)(3-phenoxybenzyl)amino)acetate (19)—A solution of t-butyl alcohol (2.43 g; 32.8 mmol) in 20 mL CH_2Cl_2 was added to a solution of chlorosulfonyl isocyanate (4.74 g; 32.8 mmol) in 20 mL CH_2Cl_2 at 0 °C. The resulting solution was added dropwise to a solution compound ***18*** (10.09 g; 32.8 mmol) in CH_2Cl_2 (80 mL) and the reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with 5% HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was removed, leaving a crude product which was purified by flash chromatography (silica gel/ethyl acetate/ hexanes) to give compound ***19*** as a white solid (6.30 g; 43% yield). ^1H NMR ($CDCl_3$): δ 1.50 (s, 9H), 3.76 (s, 3H), 4.02 (s, 2H), 4.60 (s, 2H), 6.90–7.18 (m, 6H), 7.21–7.39 (m, 4H).

Methyl 2-((3-phenoxybenzyl)(sulfamoyl)amino)acetate (20)—To compound ***19*** (10.9 g; 22.2 mmol) was added 60 mL trifluoroacetic acid and the reaction mixture was stirred at room temperature overnight. The trifluoroacetic acid was removed and the residue was taken up in ethyl acetate (100 mL) and washed with saturated sodium bicarbonate (3 × 50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and the solvent was removed to yield compound ***20*** as yellow oil (7.60 g; 99% yield). ^1H NMR ($CDCl_3$): δ 3.75 (s, 3H), 3.98 (s, 2H), 4.20 (s, 2H), 5.09 (s, 2H), 6.90–7.00 (m, 4H), 7.05–7.18 (m, 2H), 7.23–7.40 (m, 3H).

5-(3-Phenoxybenzyl)-1,2,5-thiadiazolidin-3-one 1,1-dioxide (21)—To a solution of compound ***20*** (7.6 g; 21.68 mmol) in dry THF (50 mL) cooled in an ice bath, was added sodium hydride (1.15 g; 60% w/w ; 28.8 mmol) in small portions and the reaction mixture was stirred at room temperature overnight. The solvent was removed, water (100 mL) was added water to the residue and the pH of the solution was adjusted to ~1. The aqueous layer was extracted with ethyl acetate (2 × 75 mL) and the organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent removed. The crude product was purified by flash chromatography (silica gel/ethyl acetate/ hexanes) to give compound ***21*** as yellow oil (6.75 g; 100% yield). ^1H NMR ($CDCl_3$): δ 3.82 (s, 2H), 4.37 (s, 2H), 4.90–5.20 (s, 1H), 6.92–7.18 (m, 6H), 7.22–7.40 (m, 3H). HRMS (ESI) calculated m/z $C_{15}H_{13}N_2O_4S$ $[M-H]^+$ 317.0596; found 317.0495.

Biochemical studies

One-day old, 80–90% confluent HG23 cells were treated with varying concentrations of each compound (0 [mock-DMSO]- 20 μM) to examine its effects on the replication of NV. At 24 or 48 hrs of treatment, the NV genome was analyzed with qRT-PCR. The ED_{50}s of the compounds for NV genome levels were determined at 24 hr post-treatment. The cytotoxic effects of the compounds on HG23 cells were determined with varying

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concentrations of each compound (0 [mock-DMSO]-320 μM) using a cell cytotoxicity assay kit (Promega, Madison, WI) to calculate the median toxic dose (TD_{50}) at 48 hr of treatment.

Acknowledgments

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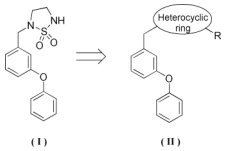
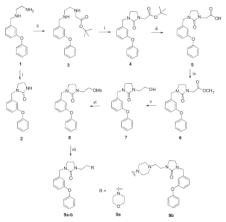


Figure 1.
Norovirus inhibitor scaffold hopping strategy.



Scheme 1.

Reaction conditions: i) CDI/1,4-dioxane; ii) $BrCH_2COOC(CH_3)_3$/DMF/0°C to rt; iii) TFA; iv) $SOCl_2/CH_3OH$; v) $LiBH_4$/THF/EtOH; vi) MsCl/TEA/CH_2Cl_2; vii) 1eq morpholine or 0.5 eq piperazine, $NaHCO_3$/95% EtOH/reflux.

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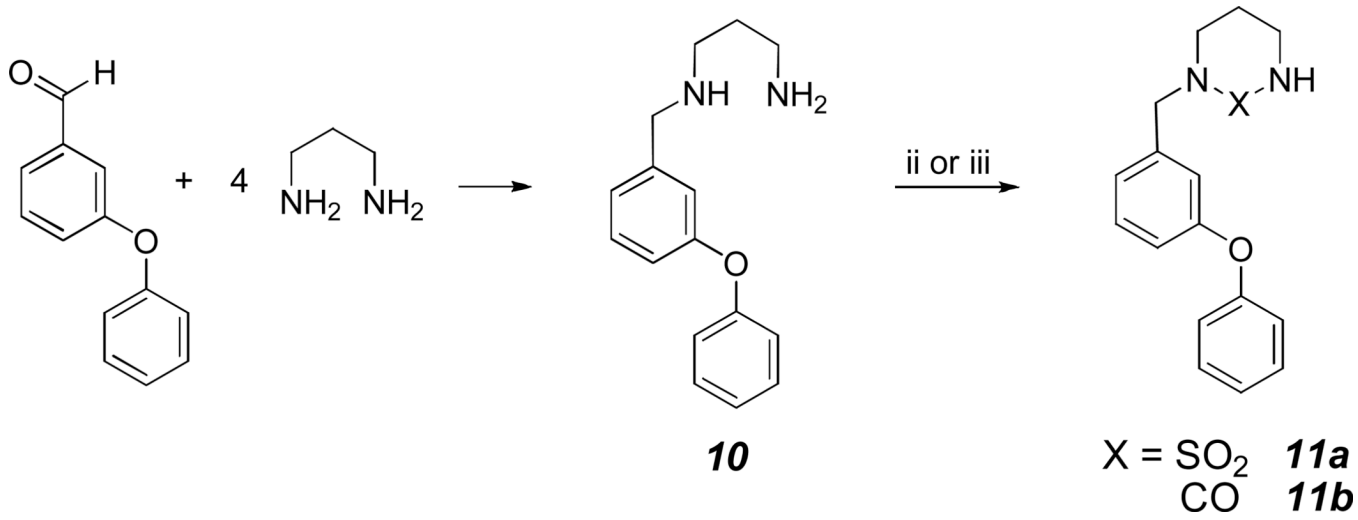

Scheme 2.

Reaction conditions: i) $NaBH_4$/MeOH; ii) $NH_2SO_4NH_2$/pyridine/reflux 16h; iii) CDI/1,4-dioxance

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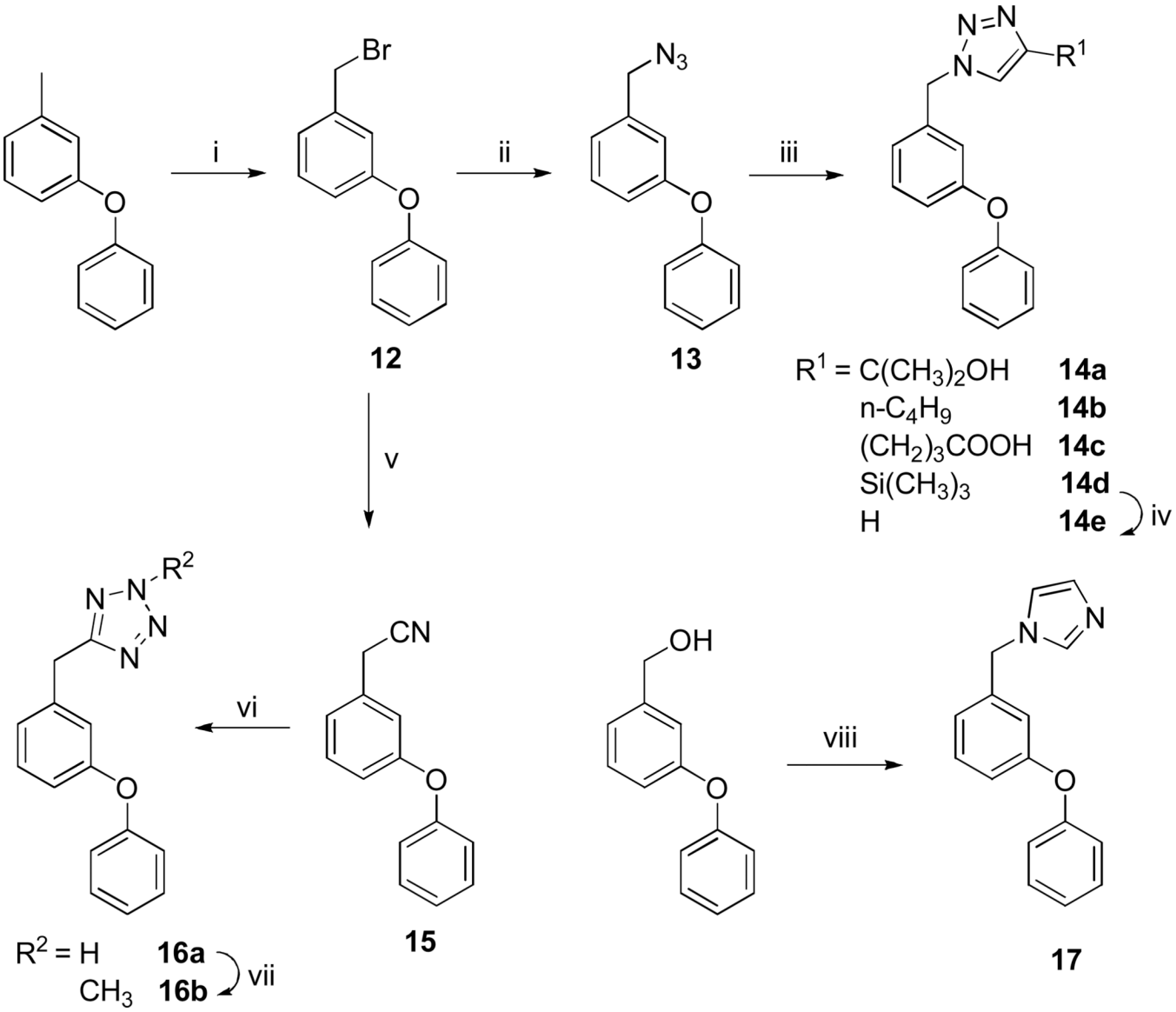

Scheme 3.

Reaction conditions: i) NBS/AIBN/CCl_4; ii) NaN_3/DMSO; iii) $R^1C{\equiv}CH$/Sodium ascorbate/$CuSO_4$/t-BuOH:H_2O(1:1) or CuI/DMSO; iv) TBAF/THF; v) NaCN/DMSO; vi) NaN_3/NH_4Cl/DMF/100 °C; vii) CH_3I/TEA/ACN; viii) CDI/THF/ACN.

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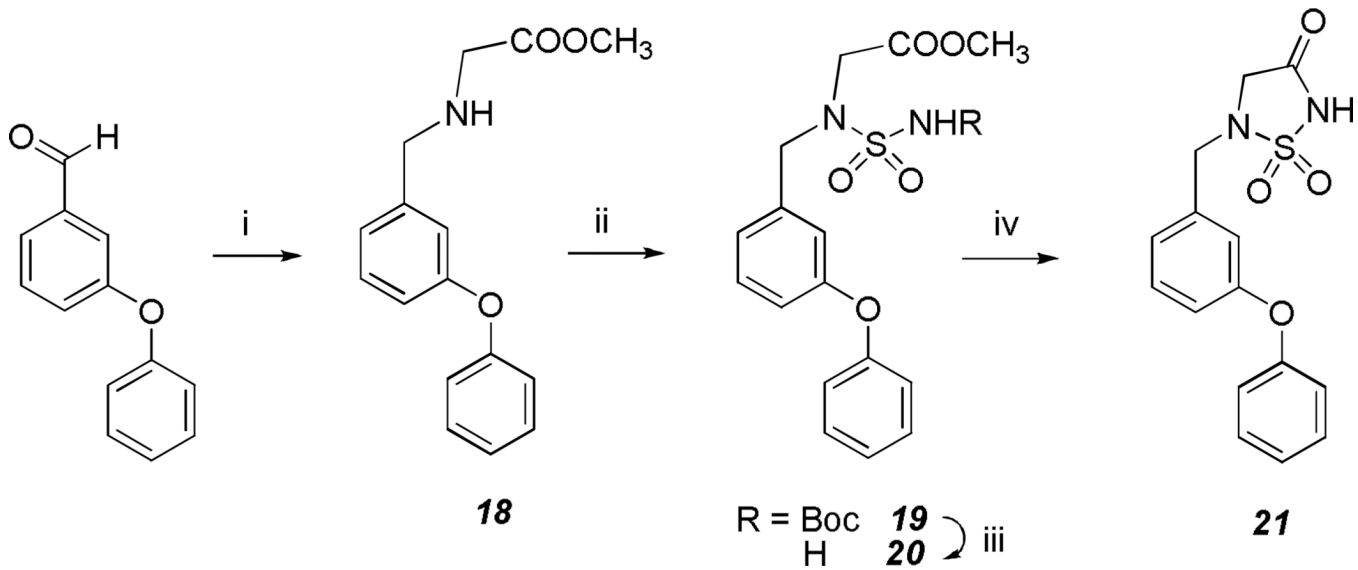

Scheme 4.

Reaction conditions: i) Gly-OCH_3(HCl)/TEA/$NaBH_4$/CH_3OH; ii) a) $ClSO_2NCO$/t-BuOH/CH_2Cl_2, b) TEA/CH_2Cl_2; iii) TFA; iv) NaH/THF.

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Table 1

Compound	ED_{50} (μM)	TD_{50} (μM)
2	8	95
4	12	55
5	>20	ND
9a	16	120
9b	0.5	2.5
11a	> 20	ND
11b	> 20	ND
14a	> 20	ND
14b	6	40
14c	> 20	ND
14d	7	30
14e	10	100
16a	> 20	ND
16b	> 20	ND
17	8	35
21	>20	ND

*ND: not determined due to high ED_{50} value