

## **SMALL MOLECULE INHIBITORS OF BACTERIAL TOXINS**

### **CROSS-REFERENCE TO RELATED APPLICATIONS**

**[0001]** This application claims the benefit of priority to U.S. Provisional Application No. 63/077,354, filed September 11, 2020, which is hereby incorporated by reference in its entirety.

### **FIELD OF THE DISCLOSURE**

**[0002]** The present disclosure relates to compounds, compositions and methods for treating gastrointestinal diseases such as inflammatory bowel disease and gastrointestinal cancer. The present disclosure also relates to small molecule compounds, and compositions comprising the same, which bind to and/or inhibit toxins produced by various pathogenic bacterial strains.

### **SEQUENCE LISTING**

**[0003]** This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "ARTI\_006\_01WO\_SeqList\_ST25.txt" created on September 9, 2020 and having a size of ~38.9 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

### **BACKGROUND**

**[0004]** Inflammatory bowel disease (IBD) is a group of inflammatory diseases of the colon and small intestine, including Crohn's disease and colitis. The most common forms of IBD are Crohn's disease and ulcerative colitis. Ulcerative colitis affects the large intestine (colon) and rectum and involves the inner lining (e.g., the mucosal and sub-mucosal layer) of the intestinal wall. Crohn's disease may affect any section of the gastrointestinal tract (e.g., mouth, esophagus, stomach, small intestine, large intestine, rectum, anus, etc.) and may involve all layers of the intestinal wall. The clinical symptoms of IBD include rectal and/or intestinal bleeding, abdominal pain and cramping, diarrhea, and weight loss. In

addition, IBD is a risk factor for colon cancer, and this risk for colon cancer increases significantly after eight to ten years of IBD.

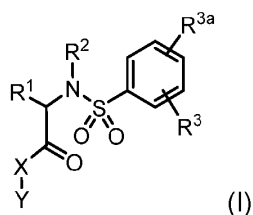
**[0005]** Although the etiology of IBD is unclear, experiments in animal models and humans have suggested that commensal bacteria play an important role in the pathogenesis of IBD. However, the exact nature of host-microbe interactions that contribute to IBD development is still unknown. Bacteria may contribute to IBD, for example, as causative agents, or may simply contribute to the perpetuation of the disease. Understanding bacterial functions in IBD can identify potential therapeutic approaches.

**[0006]** There is no cure for IBD, and currently available treatments do not work for all patients. Accordingly, there is a need in the art for improved compositions and methods for treating IBD.

## SUMMARY

**[0007]** The present disclosure is directed to compounds and compositions thereof that inhibit the activity of one or more pathogenic bacterial toxins, such as *B. fragilis* toxin (BFT), collagenase A (ColA) and gelatinase E (GelE). The disclosed compounds and compositions are useful in treating various diseases and disorders including inflammatory bowel disease, gastrointestinal cancer, and systemic bacterial infections in subjects in need thereof.

**[0008]** In some embodiments, the present disclosure provides a compound of Formula I:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-5} \text{ alkyl})-$ , or  $-\text{N}(\text{CH}_2\text{CF}_3)-$ , Y is  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{OCH}_2\text{CH}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{CH}_3)$ ,  $-\text{NH}(\text{CH}_2\text{CH}_3)$ , or  $-\text{NH}(\text{CH}_2\text{CF}_3)$ ; and

when X is  $-\text{O}-$ , Y is H;

$\text{R}^1$  is alkyl, aryl, -alkylene-OH, -alkylene- $\text{NH}_2$ , -alkylene- $\text{C}(=\text{O})\text{NH}_2$ , heteroaralkyl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein  $\text{R}^1$  is optionally substituted with one or more groups selected from  $-\text{OH}$ ,  $-\text{OMe}$ , halogen,  $-\text{CHF}_2$ ,  $-\text{CH}_2\text{F}$ , or  $-\text{CF}_3$ ;

$\text{R}^2$  is H,  $-(\text{CH}_2)_n\text{-aryl}$ ,  $-\text{CH}_2\text{-alkyl}$ ,  $-\text{CH}(\text{Me})\text{-alkyl}$ ,  $-\text{CH}_2\text{-heterocyclyl}$ ,  $-(\text{CH}_2)_n\text{-heteroaryl}$ , or  $\text{CH}_2\text{-haloalkyl}$ ; and

$\text{R}^3$  is F,  $-\text{OH}$ , alkoxy,  $-\text{O-alkylene-NR}^5\text{R}^6$ , alkyl,  $-\text{S-alkyl}$ ,  $-\text{NH-alkyl}$ ,  $-\text{N}(\text{CH}_3)\text{-alkyl}$ ,  $-\text{N}(\text{H})\text{aralkyl}$ ,  $-\text{N}(\text{H})\text{C}(\text{O})\text{-alkylene-NR}^5\text{R}^6$ ,  $-\text{O-haloalkyl}$ ,  $-\text{O-aryl}$ ,  $-\text{O-heteroaryl}$ , or  $-\text{O-aralkyl}$ , each of which is optionally substituted;

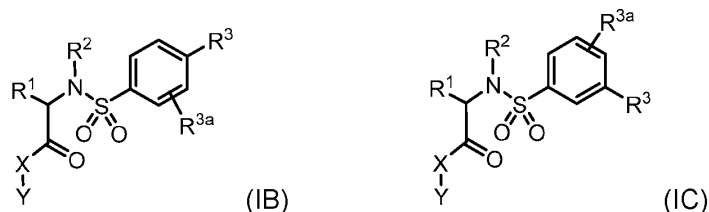
$\text{R}^{3a}$  is H or halogen;

$\text{R}^5$  is H,  $-\text{C}(\text{O})\text{alkyl}$ ,  $-\text{C}(\text{O})\text{aryl}$ ,  $-\text{C}(\text{O})\text{heteroaryl}$ , or  $-\text{C}(\text{O})\text{aralkyl}$ ;

$\text{R}^6$  is H, alkyl, or aryl; and

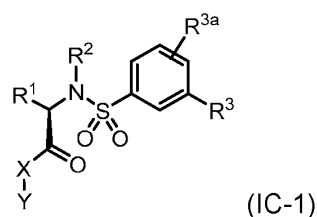
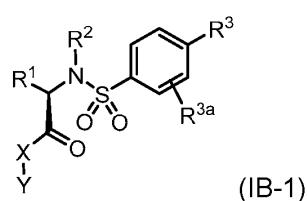
n is an integer from 0 to 3.

**[0009]** In some embodiments, the compound of Formula I is a compound of Formula IB or Formula IC:



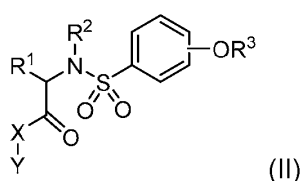
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y,  $\text{R}^1$ ,  $\text{R}^2$ ,  $\text{R}^3$ , and  $\text{R}^{3a}$  are as defined herein for Formula (I).

**[0010]** In some embodiments, the compound of Formula I is a compound of Formula IB-1 or Formula IC-1:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, and R<sup>3a</sup> are as defined herein for Formula (I).

**[0011]** In some embodiments, the present disclosure provides a compound of Formula II:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is -NH-, -N(C<sub>1-5</sub> alkyl)-, or -N(CH<sub>2</sub>CF<sub>3</sub>)-, Y is -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>); and

when X is -O-, Y is H;

R<sup>1</sup> is alkyl, aryl, -alkylene-OH, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, heteroaralkyl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein R<sup>1</sup> is optionally substituted with one or more groups selected from -OH, -OMe, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>;

R<sup>2</sup> is H, -(CH<sub>2</sub>)<sub>n</sub>-aryl, -CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or CH<sub>2</sub>-haloalkyl; and

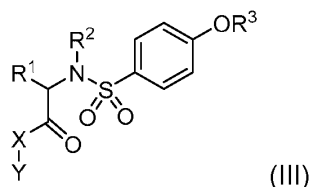
R<sup>3</sup> is H, alkyl, -alkylene-NR<sup>5</sup>R<sup>6</sup>, haloalkyl, aryl, heteroaryl, or aralkyl, each of which is optionally substituted;

R<sup>5</sup> is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl;

R<sup>6</sup> is H, alkyl, or aryl; and

n is an integer from 0-3.

**[0012]** In some embodiments, the present disclosure provides a compound of Formula III:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{-NH-}$ ,  $\text{-N(C}_{1-5}\text{ alkyl)-}$ ,  $\text{-N(CH}_2\text{CF}_3\text{)-}$ , or  $\text{-O-}$ ;

Y is  $\text{-OH}$ ,  $\text{-OCH}_3$ ,  $\text{-OCH}_2\text{CH}_3$ ,  $\text{-NH}_2$ ,  $\text{-NH(CH}_3\text{)}$ ,  $\text{-NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{-NH(CH}_2\text{CF}_3\text{)}$ ;

$\text{R}^1$  is alkyl, aryl,  $\text{-alkylene-OH}$ ,  $\text{-alkylene-NH}_2$ ,  $\text{-alkylene-C(=O)NH}_2$ , heteroaralkyl, aralkyl,  $\text{-alkylene-S-alkyl}$ ,  $\text{-alkylene-S-haloalkyl}$ ,  $\text{-alkylene-S-aralkyl}$ , or  $\text{-alkylene-S-heteroaralkyl}$ ; wherein  $\text{R}^1$  is optionally substituted with one or more groups selected from  $\text{-OH}$ , halogen,  $\text{-CHF}_2$ ,  $\text{-CH}_2\text{F}$ , or  $\text{-CF}_3$ ;

$\text{R}^2$  is H,  $\text{-(CH}_2\text{)}_n\text{-aryl}$ ,  $\text{-CH}_2\text{-alkyl}$ ,  $\text{-CH(Me)-alkyl}$ ,  $\text{-(CH}_2\text{)}_n\text{-heteroaryl}$ , or  $\text{CH}_2\text{-haloalkyl}$ ; and

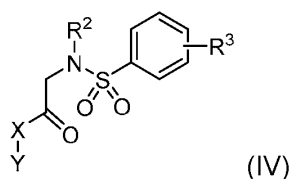
$\text{R}^3$  is H, alkyl,  $\text{-alkylene-NR}^5\text{R}^6$ , haloalkyl, aryl, heteroaryl, or aralkyl, each of which is optionally substituted;

$\text{R}^5$  is H,  $\text{-C(O)alkyl}$ ,  $\text{-C(O)aryl}$ ,  $\text{-C(O)heteroaryl}$ , or  $\text{-C(O)aralkyl}$ ;

$\text{R}^6$  is H, alkyl, or aryl; and

n is an integer from 0 to 3.

**[0013]** In some embodiments, the present disclosure provides a compound of Formula IV:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{-NH-}$ ,  $\text{-N(C}_{1-5}\text{ alkyl)-}$ ,  $\text{-N(CH}_2\text{CF}_3\text{)-}$ , or  $\text{-O-}$ ;

Y is H,  $\text{-OH}$ ,  $\text{-OCH}_3$ ,  $\text{-OCH}_2\text{CH}_3$ ,  $\text{-NH}_2$ ,  $\text{-NH(CH}_3\text{)}$ ,  $\text{-NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{-NH(CH}_2\text{CF}_3\text{)}$ ;

wherein:

when X is  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-5} \text{ alkyl})-$ , or  $-\text{N}(\text{CH}_2\text{CF}_3)-$ , Y is  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{OCH}_2\text{CH}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{CH}_3)$ ,  $-\text{NH}(\text{CH}_2\text{CH}_3)$ , or  $-\text{NH}(\text{CH}_2\text{CF}_3)$ ; and

when X is  $-\text{O}-$ , Y is H;

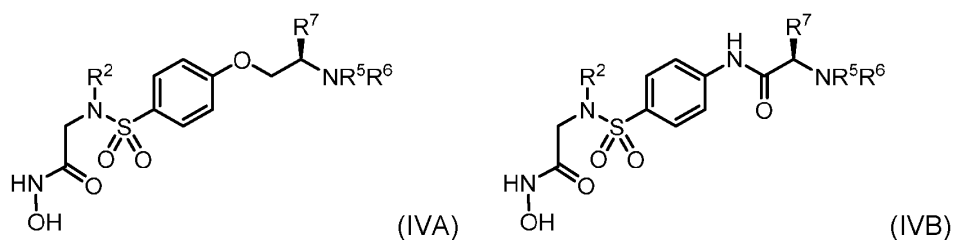
$\text{R}^2$  is  $-\text{CH}_2\text{-aryl}$ ,  $-\text{CH}_2\text{-alkyl}$ ,  $-\text{CH}(\text{Me})\text{-alkyl}$ ,  $-\text{CH}_2\text{-heteroaryl}$ , or  $-\text{CH}_2\text{-haloalkyl}$ ;

$\text{R}^3$  is  $-\text{OH}$ , alkoxy,  $-\text{O-alkylene-NR}^5\text{R}^6$ , alkyl,  $-\text{S-alkyl}$ ,  $-\text{NH-alkyl}$ ,  $-\text{N}(\text{CH}_3)\text{-alkyl}$ ,  $-\text{N}(\text{H})\text{aralkyl}$ ,  $-\text{N}(\text{H})\text{C}(\text{O})\text{-alkylene-NR}^5\text{R}^6$ ,  $-\text{O-haloalkyl}$ ,  $-\text{O-aryl}$ ,  $-\text{O-heteroaryl}$ , or  $-\text{O-aralkyl}$ , each of which is optionally substituted;

$\text{R}^5$  is H, alkyl, aralkyl, heteroaralkyl,  $-\text{C}(\text{O})\text{alkyl}$ ,  $-\text{C}(\text{O})\text{aryl}$ ,  $-\text{C}(\text{O})\text{heteroaryl}$ , or  $-\text{C}(\text{O})\text{aralkyl}$ ; and

$\text{R}^6$  is H, alkyl, or aryl.

**[0014]** In some embodiments, the compound of Formula IV is a compound of Formula IVA or Formula IVB:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

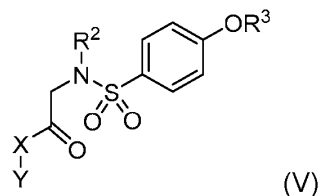
$\text{R}^2$  is alkyl;

$\text{R}^5$  is H, aralkyl, heteroaralkyl,  $-\text{C}(\text{O})\text{alkyl}$ ,  $-\text{C}(\text{O})\text{aryl}$ ,  $-\text{C}(\text{O})\text{heteroaryl}$ , or  $-\text{C}(\text{O})\text{aralkyl}$ ; and

$\text{R}^6$  is H, alkyl, or aryl; and

$\text{R}^7$  is F, oxo, alkyl, fluoroalkyl, aryl,  $-\text{CH}_2\text{-aryl}$ , or  $-\text{CH}_2\text{-heteroaryl}$ .

**[0015]** In some embodiments, the present disclosure provides a compound of Formula V:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{--NH--}$ ,  $\text{--N(C}_{1-5}\text{ alkyl)--}$ ,  $\text{--N(CH}_2\text{CF}_3\text{)--}$ , or  $\text{--O--}$ ;

Y is H,  $\text{--OH}$ ,  $\text{--OCH}_3$ ,  $\text{--OCH}_2\text{CH}_3$ ,  $\text{--NH}_2$ ,  $\text{--NH(CH}_3\text{)}$ ,  $\text{--NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{--NH(CH}_2\text{CF}_3\text{)}$ ;

wherein:

when X is  $\text{--NH--}$ ,  $\text{--N(C}_{1-5}\text{ alkyl)--}$ , or  $\text{--N(CH}_2\text{CF}_3\text{)--}$ , Y is  $\text{--OH}$ ,  $\text{--OCH}_3$ ,  $\text{--OCH}_2\text{CH}_3$ ,  $\text{--NH}_2$ ,  $\text{--NH(CH}_3\text{)}$ ,  $\text{--NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{--NH(CH}_2\text{CF}_3\text{)}$ ; and

when X is  $\text{--O--}$ , Y is H;

$\text{R}^2$  is H,  $\text{--CH}_2\text{--aryl}$ ,  $\text{CH}_2\text{--alkyl}$ ,  $\text{--CH(Me)--alkyl}$ ,  $\text{--CH}_2\text{--heteroaryl}$ , or  $\text{--CH}_2\text{--haloalkyl}$ ;

and

$\text{R}^3$  is H, alkyl,  $\text{--alkylene--NR}^5\text{R}^6$ , haloalkyl, aryl, aralkyl, or heteroaryl, each of which is optionally substituted;

$\text{R}^5$  is H, alkyl, aralkyl, heteroaralkyl,  $\text{--C(O)alkyl}$ ,  $\text{--C(O)aryl}$ ,  $\text{--C(O)heteroaryl}$ , or  $\text{--C(O)aralkyl}$ ; and

$\text{R}^6$  is H, alkyl, or aryl.

**[0016]** In some embodiments, the present disclosure provides a pharmaceutical composition comprising a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA and a pharmaceutically acceptable carrier or excipient.

**[0017]** Provided herein are methods of treating inflammatory bowel disease in a subject in need thereof, the method comprising, administering to the subject a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA). In some embodiments, the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

**[0018]** Also provided herein are methods of treating gastrointestinal cancer in a subject in need thereof, the methods comprising, administering to the subject a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA). In some embodiments, the GI cancer is esophageal cancer, gallbladder cancer, liver cancer,

pancreatic cancer, stomach cancer, cancer of the small intestine, colorectal cancer, or anal cancer.

**[0019]** Also provided herein are methods of treating a systemic bacterial infection in a subject in need thereof, the methods comprising, administering to the subject a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, or Formula V). In some embodiments, the systemic bacterial infection is endocarditis or a urinary tract infection.

**[0020]** In some embodiments, the subject is colonized by one or more pathogenic bacterial strains. In some embodiments, the pathogenic bacterial strain is *B. fragilis*, *E. faecalis*, and/or *C. perfringens*. In some embodiments, the pathogenic bacterial strain is a strain of *B. fragilis* expressing the BFT toxin. In some embodiments, the pathogenic bacterial strain is a strain of *E. faecalis* expressing the gelatinase GelE. In some embodiments, the pathogenic bacterial strain is a strain of *C. perfringens* expressing the collagenase ColA.

**[0021]** In some embodiments, administering a compound of the present disclosure reduces and/or eliminates the activity of at least one of BFT, ColA and/or GelE in the subject. In some embodiments, administering the compound reduces and/or eliminates the activity of BFT in the subject. In some embodiments, administering the compound results in a reduction in the number of *B. fragilis*, *E. faecalis*, and/or *C. perfringens* in the subject.

**[0022]** In some embodiments, a compound of the present disclosure binds to and/or inhibits one or more of *B. fragilis* toxin (BFT), collagenase A (ColA), and gelatinase E (GelE). In some embodiments, the compound binds to BFT, ColA, and/or GelE with an inhibition constant ( $K_i$ ) in the range of about  $10^{-5}$  M to about  $10^{-13}$  M. In some embodiments, the BFT comprises the amino acid sequence of any one of SEQ ID NO: 2-4. In some embodiments, the BFT comprises an amino acid sequence that is at least 90%, at least 95%, or at least 98% identical to any one of SEQ ID NO: 2-4. In some embodiments, the GelE comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the GelE comprises an amino acid sequence that is at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 6. In some embodiments, the ColA

comprises the amino acid sequence of SEQ ID NO: 8. In some embodiments, the CoIA comprises an amino acid sequence that is at least 90%, at least 95%, or at least 98% identical to SEQ ID NO: 8.

**[0023]** In some embodiments, the compound is administered intravenously to the subject. In some embodiments, the compound is administered orally to the subject. In some embodiments, the compound is administered in a tablet or a capsule, wherein the tablet or capsule optionally comprises a pharmaceutically acceptable carrier or excipient. In some embodiments, the compound is administered as a liquid formulation, wherein the liquid formulation optionally comprises a pharmaceutically acceptable carrier or excipient.

**[0024]** In some embodiments, the compound is administered once per day, once per week, or multiple times per day or week. In some embodiments, the dose of the compound administered to the subject is from about 0.001 to about 1000 mg/kg of body weight per day.

**[0025]** These and other aspects are described in greater detail below.

### BRIEF DESCRIPTION OF THE DRAWINGS

**[0026] FIG. 1** shows a crystal structure of BFT, a zinc-dependent metalloprotease. The inset shows the zinc-binding domain. BFT is produced by the cell as an inactive protease comprising an inhibitory pro-domain which inserts itself into the active site of the enzyme to inhibit toxin activity. The pro-domain is cleaved by a protease (e.g., fragipain or other host proteases such as trypsin) to produce an active toxin. **FIG. 1** is adapted from Goulas, *et al.*, PNAS (2010).

**[0027] FIG. 2** shows a schematic of the cell-based BFT toxicity assay for screening test compounds. Recombinant BFT is pre-incubated with one or more test compounds. The BFT-inhibitor mixture is applied to a cell monolayer. After 18 hours of incubation at 37°C, cellular supernatants are collected. The activity of BFT may be quantified by measuring E-cadherin or IL-8 levels in the supernatant, for example using a standard ELISA.

**[0028] FIG. 3A** is a chemical structure for 2(R)-[4-hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrohydroxamic acid (OH-CGS-27023A). **FIG. 3B** is a chemical structure for 2(R)-[4-methoxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrohydroxamic acid (CGS-27023A).

**[0029] FIG. 4** shows percent inhibition of E-cadherin release (a measure of BFT activity) following treatment with 25 pM to 50  $\mu$ M of OH-CGS-27023A.

**[0030] FIG. 5A** shows hydrolysis of NFF-3 following treatment with different concentrations of BFT at varying concentrations of NFF-3 substrate. **FIG. 5B** shows percent inhibition of NFF-3 hydrolysis following treatment with 5.65 nM to 1 mM of 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid (OH-CGS-27023A).

**[0031] FIG. 6A** is a schematic of the ETBF-mediated disease model for screening test compounds *in vivo*. Germ-free (GF) mice were mono-colonized with ETBF on day 0. On days 1, 2, and 3 following colonization, mice were orally administered 50 mg/kg of the test compound two times per day (BID). Markers of inflammation were analyzed on day 4. **FIG. 6B** shows cecal weight in mice mono-colonized with ETBF following treatment with 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid (OH-CGS-23270A) or vehicle control as described in **FIG. 6A**. **FIG. 6C** shows fecal lipocalin2 (Lcn2) in mice mono-colonized with ETBF following treatment for 3 days with 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid (OH-CGS-23270A) or vehicle control as described in **FIG. 6A**. \*\*\*p=0.0002; \*\*\*\*p<0.0001.

**[0032] FIG. 7** shows percent inhibition of GelE activity following treatment with 714 pM to 200  $\mu$ M of Compound A.

## DETAILED DESCRIPTION

**[0033]** Provided herein are compounds, e.g., small molecule inhibitors of BFT, GelE, and/or ColA, that are useful in treating a disease or disorder in subject in need thereof. In some embodiments, the disease or disorder is an inflammatory bowel disease, gastrointestinal cancer, or a systemic bacterial infection and the subject is colonized by one or more pathogenic bacterial strains, e.g., *B. fragilis*, *E. faecalis*, and/or *C. perfringens*.

**[0034]** As described herein, *B. fragilis*, *E. faecalis* and *C. perfringens* have been identified as causative agents that contribute to the development and progression of inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease, and may therefore

be targeted in the prevention and/or treatment thereof. Strains of each of these three bacterial species produce toxins (BFT from *B. fragilis*, GelE from *E. faecalis*, and ColA from *C. perfringens*) that are believed to contribute to the pathogenesis of IBD, and are therefore therapeutic targets. Compounds of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, or Formula V) bind and/or inhibit the activity of these toxins *in vitro* and/or *in vivo*, and may therefore be used to treat or prevent IBD and other gastrointestinal diseases in subjects in need thereof.

### **Definitions**

**[0035]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are described.

**[0036]** As used herein, each of the following terms has the meaning associated with it in this section.

**[0037]** The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

**[0038]** "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of  $\pm 20\%$  or  $\pm 10\%$ , more preferably  $\pm 5\%$ , even more preferably  $\pm 1\%$ , and still more preferably  $\pm 0.1\%$  from the specified value, as such variations are appropriate to perform the disclosed methods.

**[0039]** A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate. In contrast, a "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

**[0040]** The term “treating” as used herein with regard to a patient, refers to improving at least one symptom of the patient’s disorder. Treating can be improving, or at least partially ameliorating a disorder. For purposes of the present disclosure, treating includes, but is not limited to improving, or at least partially ameliorating the effects of IBD, gastrointestinal cancer, a systemic bacterial infection and related conditions.

**[0041]** The terms “administer,” “administering” or “administration” as used herein refer to either directly administering a compound or pharmaceutically acceptable salt or ester of the compound or a composition comprising the compound or pharmaceutically acceptable salt or ester of the compound to a patient.

**[0042]** A disease or disorder is “alleviated,” “ameliorated” or “improved” if the severity of a sign or symptom of the disease or disorder, the frequency with which such a sign or symptom is experienced by a patient, or both, is reduced.

**[0043]** An “effective amount” or “therapeutically effective amount” of a compound is that amount of a compound which is sufficient to provide a beneficial effect to the subject to which the compound is administered.

**[0044]** The terms “patient,” “subject,” “individual,” and the like are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in vivo*, amenable to the methods described herein. In certain non-limiting embodiments, the patient, subject or individual is, by way of non-limiting examples, a human, a dog, a cat, a horse, or other domestic mammal.

**[0045]** As used herein, a “pharmaceutical composition” is meant to encompass a composition suitable for administration to a subject, such as a mammal, especially a human. In general a “pharmaceutical composition” is sterile, and is usually free of contaminants that are capable of eliciting an undesirable response within the subject (e.g., the compound(s) in the pharmaceutical composition is (are) pharmaceutical grade). Pharmaceutical compositions can be designed for administration to subjects or patients in need thereof via a number of different routes of administration including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, intratracheal and the like.

**[0046]** The phrase “pharmaceutically acceptable excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable, and includes an excipient, that is

acceptable for veterinary use as well as human pharmaceutical use. "A pharmaceutically acceptable excipient" as used in the specification and claims includes both one and more than one such excipient.

**[0047]** In the context of the present disclosure, the following abbreviations for the commonly occurring nucleic acid bases are used. "A" refers to adenosine, "C" refers to cytosine, "G" refers to guanosine, "T" refers to thymidine, and "U" refers to uridine. The term "polynucleotide" as used herein is defined as a chain of nucleotides. Furthermore, nucleic acids are polymers of nucleotides. Thus, nucleic acids and polynucleotides as used herein are interchangeable. One skilled in the art has the general knowledge that nucleic acids are polynucleotides, which can be hydrolyzed into the monomeric "nucleotides." The monomeric nucleotides can be hydrolyzed into nucleosides. As used herein polynucleotides include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, i.e., the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR, and the like, and by synthetic means.

**[0048]** The term "small molecule" generally refers to a compound having a molecular weight less than or equal to 700 daltons. In some embodiments, a "small molecule" has a molecular weight less than or equal to 600 daltons, 500 daltons, or 400 daltons, or 300 daltons. In some embodiments, a "small molecule" has a molecular weight less than or equal to about 400 daltons. In some embodiments, a "small molecule" has a molecular weight less than or equal to about 300 daltons. In the present disclosure, the term "small molecule" may be used interchangeably with "compound" or "compound of the present disclosure" or any other term that refers to a compound of the present disclosure without altering meaning.

**[0049]** The term "amino acid" includes, but is not limited to, the group comprising of alanine (Ala or A), cysteine (Cys or C), aspartic acid (Asp or D), glutamic acid (Glu or E), phenylalanine (Phe or F), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), lysine (Lys or K), leucine (Leu or L), methionine (Met or M), asparagine (Asn or N), proline (Pro or P), glutamine (Gln or Q), arginine (Arg or R), serine (Ser or S), threonine (Thr or T), valine (Val or V), tryptophan (Trp or W), and tyrosine (Tyr or Y) residues. The terms

"peptide", "polypeptide", and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds.

**[0050]** The term "alkyl" as used herein refers to a branched or straight chain alkyl, wherein alkyl chain length is indicated by a range of numbers. In some embodiments, "straight chain alkyl" refers to an alkyl chain as defined above containing 1, 2, 3, 4, 5, or 6 carbons (i.e., C1 -C6 alkyl). Examples of a straight chain alkyl group include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, and hexyl. In some embodiments, "branched alkyl" refers to an alkyl chain as defined above containing from 3, 4, 5, 6, 7, or 8 carbons (i.e., branched C3-C8 alkyl). Examples of a branched alkyl group include, but are not limited to, isopropyl, isobutyl, secondary-butyl, tertiary- butyl, isoamyl, and isopentyl. Unless stated otherwise specifically in the specification, an alkyl group can be optionally substituted.

**[0051]** The term "alkoxy" as used herein refers to -O-(alkyl), wherein "alkyl" is as defined above as a branched or straight chain alkyl. Unless stated otherwise specifically in the specification, an alkoxy group can be optionally substituted.

**[0052]** The term "alkylene" as used herein refers to a divalent alkyl moiety interposed between two other atoms. In exemplary embodiments, "alkylene" refers to an alkyl moiety as defined above containing 1, 2, or 3 carbons. Examples of an alkylene group include, but are not limited to -CH<sub>2</sub>-, -CH<sub>2</sub>CH<sub>2</sub>-, and -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-. In exemplary embodiments, alkylene groups are branched. Unless stated otherwise specifically in the specification, an alkylene group can be optionally substituted.

**[0053]** The term "aryl" as used herein refers to a cyclic hydrocarbon, where the ring is characterized by delocalized  $\pi$  electrons (aromaticity) shared among the ring members, and wherein the number of ring atoms is indicated by a range of numbers. In exemplary embodiments, "aryl" refers to a cyclic hydrocarbon as described above containing 6, 7, 8, 9, or 10 ring atoms (i.e., C<sub>6</sub>-C<sub>10</sub> aryl). Examples of an aryl group include, but are not

limited to, benzene, naphthalene, tetralin, indene, and indane. Unless stated otherwise specifically in the specification, an aryl group can be optionally substituted.

**[0054]** The term "aralkyl" as used herein means an aryl group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of aralkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, and 2-naphth-2-ylethyl. Unless stated otherwise specifically in the specification, an aralkyl group can be optionally substituted.

**[0055]** The term "haloalkyl" means an alkyl group, as defined herein, wherein at least one hydrogen is replaced with a halogen, as defined herein. Representative examples of haloalkyl include, but are not limited to, chloromethyl, 2-fluoroethyl, trifluoromethyl, difluoromethyl, fluoromethyl, pentafluoroethyl, 2,2,2-trifluoroethyl, and 2-chloro-3-fluoropentyl. In some embodiments, the haloalkyl is a C<sub>1-2</sub> fluoralkyl having from 1-5 fluorides. Non-limiting examples include CF<sub>3</sub>, CF<sub>2</sub>H, CFH<sub>2</sub>, CH<sub>2</sub>CF<sub>3</sub>, and CF<sub>2</sub>CF<sub>3</sub>. Unless stated otherwise specifically in the specification, a haloalkyl group can be optionally substituted.

**[0056]** The term "halogen" as used herein refers to fluorine, chlorine, bromine, and iodine.

**[0057]** The term "heteroaryl" as used herein refers to a cyclic ring system, wherein at least one of the ring atoms is an O, N, or S, at least one ring is aromatic, and wherein the number of ring atoms can be indicated by a range of numbers (e.g., 5- to 12-membered heteroaryl, 5- to 7-membered heteroaryl, 5-membered heteroaryl, or 6-membered heteroaryl). Heteroaryl moieties as defined herein can be bound by a single bond to other moieties via one or more C or N atoms in the ring. For example, in some embodiments, a ring N atom from the heteroaryl is the bonding atom to -C(O) to form an amide, carbamate, or urea. In exemplary embodiments, "heteroaryl" refers to a cyclic hydrocarbon as described above containing 5 or 6 ring atoms. In some embodiments, the heteroaryl is a monocyclic heteroaryl. Examples of a monocyclic heteroaryl group include, but are not limited to, pyrrole, furan, thiene, oxazole, thiazole, isoxazole, isothiazole, imidazole, pyrazole, oxadiazole, thiadiazole, triazole, tetrazole, pyridine, pyrimidine, pyrazine, pyridazine, and triazine. In some embodiments, the heteroaryl is a bicyclic heteroaryl. Examples of a bicyclic heteroaryl group include, but are not limited to, quinoline, isoquinoline, quinazoline, cinnoline, phthalazine, quinazoline, quinoxaline,

indolyl, benzoxazole, benzthiazole, and benzimidazole. Unless stated otherwise specifically in the specification, a heteraryl group can be optionally substituted.

**[0058]** The term "heteroaralkyl" as used herein means a heteroaryl, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of heteroaralkyl include, but are not limited to, pyridin-3-ylmethyl and 2-(thien-2-yl)ethyl. Unless stated otherwise specifically in the specification, a heteroaralkyl group can be optionally substituted.

**[0059]** As used herein, "pyridyl" refers to a group derived from pyridine by removal of a hydrogen atom from a ring carbon atom. In some embodiments, the pyridyl is a 3-pyridyl, 4-pyridyl, or 5-pyridyl. Unless stated otherwise specifically in the specification, a pyridyl group can be optionally substituted.

**[0060]** The term "heterocyclyl" as used herein refers to a saturated or partially unsaturated cyclic ring system wherein at least one of the ring atoms is an O, N, or S and wherein the number of ring atoms can be indicated by a range of numbers (e.g., 4- to 12-membered heterocyclyl, 4- to 7-membered heterocyclyl, 5-membered heterocyclyl, or 6-membered heterocyclyl). Heterocyclyl moieties as defined herein can be bound by a single bond to other moieties via one or more C or N atoms in the ring. For example, in some embodiments, a ring N atom from the heterocyclyl is the bonding atom to -C(O) to form an amide, carbamate, or urea. In some embodiments, the heterocyclyl ring is a monocyclic or bicyclic heterocyclyl ring. In some embodiments, the heterocyclyl ring is a monocyclic heterocyclyl ring. Non-limiting examples of heterocyclyl rings include, but are not limited to, azetidiny, pyrrolidinyl, piperidinyl, morpholinyl, thiomorpholinyl, thiolanyl, and tetrahydrofuranyl. Unless stated otherwise specifically in the specification, a heterocyclyl group can be optionally substituted.

**[0061]** The term "substituted" used herein means any of the groups described herein (e.g., alkyl, alkenyl, alkynyl, alkoxy, aryl, aralkyl, carbocyclyl, cycloalkyl, cycloalkenyl, cycloalkynyl, haloalkyl, heterocyclyl, and/or heteroaryl) wherein at least one hydrogen atom is replaced by a bond to a non-hydrogen atom such as, but not limited to: a halogen atom such as F, Cl, Br, and I; an oxygen atom in groups such as hydroxyl groups, alkoxy groups, and ester groups; a sulfur atom in groups such as thiol groups, thioalkyl groups, sulfone groups, sulfonyl groups, and sulfoxide groups; a nitrogen atom in groups such as

amines, amides, alkylamines, dialkylamines, arylamines, alkylarylamines, diarylamines, N-oxides, imides, and enamines; a silicon atom in groups such as trialkylsilyl groups, dialkylarylsilyl groups, alkylidiarylsilyl groups, and triarylsilyl groups; and other heteroatoms in various other groups. "Substituted" also means any of the above groups in which one or more hydrogen atoms are replaced by a higher-order bond (e.g., a double- or triple-bond) to a heteroatom such as oxygen in oxo, carbonyl, carboxyl, and ester groups; and nitrogen in groups such as imines, oximes, hydrazones, and nitriles. For example, "substituted" includes any of the above groups in which one or more hydrogen atoms are replaced with  $-NR_gR_h$ ,  $-NR_gC(=O)R_h$ ,  $-NR_gC(=O)NR_gR_h$ ,  $-NR_gC(=O)OR_h$ ,  $-NR_gSO_2R_h$ ,  $-OC(=O)NR_gR_h$ ,  $-OR_g$ ,  $-SR_g$ ,  $-SOR_g$ ,  $-SO_2R_g$ ,  $-OSO_2R_g$ ,  $-SO_2OR_g$ ,  $=NSO_2R_g$ , and  $-SO_2NR_gR_h$ . "Substituted" also means any of the above groups in which one or more hydrogen atoms are replaced with  $-C(=O)R_g$ ,  $-C(=O)OR_g$ ,  $-C(=O)NR_gR_h$ ,  $-CH_2SO_2R_g$ ,  $-CH_2SO_2NR_gR_h$ . In the foregoing,  $R_g$  and  $R_h$  are the same or different and independently hydrogen, alkyl, alkenyl, alkynyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, cycloalkynyl, cycloalkylalkyl, haloalkyl, haloalkenyl, haloalkynyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl. "Substituted" further means any of the above groups in which one or more hydrogen atoms are replaced by a bond to an amino, cyano, hydroxyl, imino, nitro, oxo, thioxo, halo, alkyl, alkenyl, alkynyl, alkoxy, alkylamino, thioalkyl, aryl, aralkyl, cycloalkyl, cycloalkenyl, cycloalkynyl, cycloalkylalkyl, haloalkyl, haloalkenyl, haloalkynyl, heterocyclyl, *N*-heterocyclyl, heterocyclylalkyl, heteroaryl, *N*-heteroaryl and/or heteroarylalkyl group. In addition, each of the foregoing substituents can also be optionally substituted with one or more of the above substituents.

**[0062]** Ranges: throughout this disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as

from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

### **Compounds of the Disclosure**

**[0063]** Provided herein are compounds that are useful in treating various diseases and disorders, including diseases and disorders of the gastrointestinal tract. In some embodiments, the compounds of the present disclosure are capable of inhibiting one or more toxins produced by pathogenic bacterial strains. In some embodiments, the pathogenic bacterial strain is *B. fragilis*, *E. faecalis*, and/or *C. perfringens*.

#### ***B. fragilis* and *B. fragilis* toxin (BFT)**

**[0064]** In some embodiments, the pathogenic bacterial strain is *B. fragilis*. *B. fragilis* is a gram-negative, rod-shaped bacterium, and may be identified by its 16S RNA sequence (see Table 1, below). For example, in some embodiments, a strain of *B. fragilis* has a 16S RNA sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of SEQ ID NO: 1. In some embodiments, a strain of *B. fragilis* has a 16S RNA sequence that is at least 97% identical to the sequence of SEQ ID NO: 1.

**Table 1:** Sequence encoding *B. fragilis* 16S RNA

| Species | Sequence | SEQ ID NO: |
|---------|----------|------------|
|---------|----------|------------|

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |   |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| <i>B. fragilis</i> | GCGCGATTACTAGCGAATCCAGCTTCACGAAGTCGGG<br>TTGCAGACTTCGATCCGAACTGAGAGAGGATTTTGGGA<br>TTAGCATACGGTCACCCGCTAGCTGCCTTCTGTACCCC<br>CCATTGTAACACGTGTGTAGCCCCGGACGTAAGGGCC<br>GTGCTGATTTGACGTCATCCCCACCTTCCTCACATCTT<br>ACGACGGCAGTCTCTCCAGAGTCCTCAGCATGACCTG<br>TTAGTAACTGAAGATAAGGGTTGCGCTCGTTATGGCAC<br>TTAAGCCGACACCTCACGGCACGAGCTGACGACAACC<br>ATGCAGCACCTTCACAGCGGTGATTGCTCACTGACATG<br>TTCCACATCATTCCACTGCAATTTAAGCCCGGGTAAG<br>GTTCTCGCGTATCATCGAATTAACCACATGTTCTC<br>CGCTTGTCGGGGCCCCCGTCAATTCCTTTGAGTTTCAC<br>CGTTGCCGGCGTACTCCCCAGGTGGAATACTTAATGC<br>TTTCGCTTGCCCGCTTACTGTATATCGCAAACAGCGAG<br>TATTCATCGTTTACTGTGTGGACTACCAGGGTATCTAAT<br>CCTGTTTGATACCCACACTTTCGAGCATCAGTGTCACT<br>TGCAGTCCAGTGAGCTGCCTTCGCAATCGGAGTTCTTC<br>GTGATATCTAAGCATTTACCGCTACACCACGAATTCC<br>GCCACCTCTACTGTACTCAAGACTGACAGTATCAACT<br>GCAATTTTACGGTTGAGCCGCACTTTTCAAACTGAC<br>TTACCAGTCCACCTACGCTCCCTTTAAACCCAATAAAT<br>CCGGATAACGCTCGGATCCTCCGTATTACCGCGGCTG<br>CTGGCACGGAGTTAGCCGATCCTTATTCATATAATACA<br>TACAAAACAGTATACATACTGCACTTTATTCTTATATAA<br>AAGAAGTTTACGACCCATAGAGCCTTCATCCTTCACGC<br>TACTTGCTGGTTTCAAGGCTAGCGCCCATGACCAATAT<br>TCCTCACTGCTGCCTCCCGTAGGAGTTTGGACCGTGT<br>CTCAGTTCCAATGTGGGGGACCTTCCTCTCAGAACCC<br>CTATCCATCGAAGGCTTGGTGAGCCGTTACCTCACCAA<br>CAACCTAATGGAACGCATCCCCATCCTTTACCGGAATC<br>CTTTAATAATGAAACCATGCGGAATCATTATGCTATCG<br>GGTATTAATCTTTCTTTCGAAAGGCTATCCCCGAGTAA<br>AGGGCAGGTTGGATACGTGTTACTACCCGTGCGCCG<br>GTCGCCGGCAAAGAAAGCAAGCTTTCTT | 1 |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|

**[0065]** *B. fragilis* (*Bacteroides fragilis*) is a common commensal anaerobe (about 0.5% of the human colonic flora) that shapes the host health, including the immune system. Some pathogenic strains of *B. fragilis*, including enterotoxigenic *B. fragilis* (ETBF) strains, harbor a gene encoding a pro-inflammatory enterotoxin called *B. fragilis* toxin (BFT) or fragilysin.

**[0066]** BFT, a ~20 kDa zinc-dependent metalloprotease toxin, is secreted from ETBF strains. BFT reversibly stimulates chloride secretion and alters tight junctional function in polarized intestinal epithelial cells. Experimental studies originally suggested that the

cellular target for BFT was E-cadherin, but more recent studies have suggested that BFT binds to a different, unidentified host receptor. BFT's enzymatic activity is required for ETBF's pathogenicity.

**[0067]** Enterotoxigenic strains of *B. fragilis* (*i.e.*, ETBF strains) have genes encoding a pro-inflammatory enterotoxin called BFT (FIG. 1). These strains may be differentiated from non-toxigenic strains (*i.e.*, NTBF strains) using several methods known to those of skill in the art, such as by using PCR to detect BFT genes in a *B. fragilis* sample. Exemplary ETBF strains include 86-5443-2-2, 2-078382-3, BOB25, 20656-2-1, 20793-3, 2078382-3, 20793-3, 20656-2-1, 86-5443-2-2. In some embodiments, an ETBF strain is isolated from a human fecal sample. In some embodiments, an ETBF strain is an engineered strain, such as a non-toxigenic *B. fragilis* strain engineered to express or overexpress BFT.

**[0068]** There are three known isotypes of BFT, encoded by distinct *bft* loci contained within a 6 kb chromosomal region found in ETBF strains termed the *B. fragilis* pathogenicity island (BfPAI). Various BFT isotypes are listed in Table 2, below. In some embodiments, an ETBF strain expresses at least one of BFT1, BFT2, and/or BFT3.

**Table 2:** *B. fragilis* toxin (BFT) isotypes

| Name | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                         | SEQ ID No: |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| BFT1 | MFILNFKMKNVKLLMLGTAALLAACSNEADSLTTSIDAPVTAS<br>IDLQSVSYTDLATQLNDVSDFGKMIILKDNGFNQVHVSMKRT<br>KIQLDNENVRLFNDRDKDSTSFILGDEFVLRFYRNGESISYIAY<br>KEAQMMNEIAEFYAAPFKKTRAINEKEAFECIYDSRTRSAGKDI<br>VSVKINIDKAKKILNLPECDYINDYIKTPQVPHGITESQTRAVPSE<br>PKTVYVICLRENGSTIYPNEVSAQMQDAANSVYAVHGLKRYVN<br>FHFVLYTTEYSCPSGDAKEGLEGTASLKSNPKEGYDDQIYFL<br>IRWGTWDNKILGMSWFNSYNVTASDFEASGMSTTQLMYPGV<br>MAHELGHILGAHTDNSKDLMYATFTGYLSHLSEKNMDIIAKNL<br>GWEAADGD | 2          |
| BFT2 | MKNVKLLMLGTAALLAACSNEADSLTTSIDTPVTASIDLQSVSY<br>TDLATQLNDVSDFGKMIILKDNGFNQVHVSMKRTKIQLDNE<br>NVRLFNDRDKDSTSFILGDEFVLRFYRNGESISYIAYKEAQMM<br>NEIAEFYAAPFKKTRAINEKEAFECIYDSRTRSAGKDLVSVKINID<br>KAKKILNLPECDYINDYIKTPQVPHGITESQTRAVPSEPCTVYVIC<br>LRESGSTVYPNEVSAQMQDAANSVYAVHGLKRFVNLHFVLYTT<br>EYSCPSGNADEGLDGFTASLKNPKEGYDDQIYFLIRWGTWD                                                                                                         | 3          |

|      |                                                                                                                                                                                                                                                                                                                                                                                                                |   |
|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
|      | NNILGISWLD SYNVTASDFKASGMSTTQLMYPGVMAHELGHILGARHADDPKDLMYSKYTGYLFHLSEENMYRIAKNLGWEIADGD                                                                                                                                                                                                                                                                                                                        |   |
| BFT3 | MKNVKLLMLGTAALLAACSNEADSLTTSIDAPVTASIDLQSVSYTDLATQLNDVSDFGKMIILKDNGFNQVHVSMKRTKIQLDNE NVRLFNGRDKDSTNFI LGDEFVLRFYRNGESISYIAYKEAQMM NEIAEFYAAPFKKTRAINEKEAFECIYDSRTRSAGKYPVSVKINV DKAKKILNLPECDYINDYIKTPQVPHGITESQTRAVPSEPCTVYVICLRENGSTVYPNEVSAQMQDAANSVYAVHGLKRYVNLHFVLYTTEYACPSGNADEGLDGFTASLKANPKAEGYDDQIYFLIRWGT WDNNILGISWLN SYNVTASDFKASGMSTTQLMYPGVMAHELGHILGANHADDPKDLMYSKYTGYLFHLSEKNMDIIAKNLGWEIADGD | 4 |

**[0069]** *E. faecalis* and Gelatinase E (GelE)

**[0070]** In some embodiments, the pathogenic bacterial strain is *E. faecalis*. *E. faecalis* is a gram-positive commensal bacterium, and may be identified by its 16S RNA sequence (see Table 3, below). For example, in some embodiments, a strain of *E. faecalis* has a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of SEQ ID NO: 5. In some embodiments, a strain of *E. faecalis* has a 16S RNA sequence that is at least 97% identical to the sequence of SEQ ID NO: 5. In some embodiments, an *E. faecalis* strain is isolated from a human fecal sample. In some embodiments, an *E. faecalis* strain is an engineered strain, such as a non-toxicogenic *E. faecalis* strain engineered to express or overexpress GelE.

**[0071]** Table 3: Sequence encoding *E. faecalis* 16S RNA

| Species | Sequence | SEQ ID NO: |
|---------|----------|------------|
|---------|----------|------------|

|                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |   |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| <i>E. faecalis</i> | GACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCG<br>AACGCTTCTTTCTCCCGAGTGCTTGCACTCAATTGGA<br>AAGAGGAGTGGCGGACGGGTGAGTAACACGTGGGTA<br>ACCTACCCATCAGAGGGGGATAAACAATTGGAAACAGG<br>TGCTAATACCGCATAACAGTTTATGCCGCATGGCATAA<br>GAGTGAAAGGCGCTTTTCGGGTGTCGCTGATGGATGGA<br>CCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTC<br>ACCAAGGCCACGATGCATAGCCGACCTGAGAGGGTGA<br>TCGGCCCACTGGGACTGAGACACGGCCCAGACTCCT<br>ACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACG<br>AAAGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGG<br>TTTTCGGATCGTAAACTCTGTTGTTAGAGAAGAACAA<br>GGACGTTAGTAACGAACGTCNCCTGACGGTATCTAAC<br>CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG<br>TAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGG<br>CGTAAAGCGAGCGCAGGCGGTTTCTTAAGTCTGATGT<br>GAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGA<br>CTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTC<br>CATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAA<br>CACCAGTGGCGAAGGCGGCTCTCTGGTCTGTAAGTGA<br>CGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTA<br>GATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCT<br>AAGTGTGGAGGTTTTCCGCCCTTCAGTGCTGCAGCA<br>AACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCA<br>AGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACA<br>AGCGGTGGAGCATGTGGTTTAATTGCAAGCAACGCGA<br>AGAACCTTACCAGGTCTTGACATCCTTTGACCACTCTA<br>GAGATAGAGCTTTCCCTTCGGGGACAAAGTGACAGGT<br>GGTGATGGTTGTCGTCAGCTCGTGTGTCGTGAGATGTT<br>GGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTA<br>GTTGCCATCATTTAGTTGGGCACTCTAGCGAGACTGCC<br>GGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAAT<br>CATCATGCCCCTTATGACCTGGGCTACACACGTGCTAC<br>AATGGGAAGTACAACGAGTCGCTAGACCGCGAGGTCA<br>TGCAAATCTCTTAAAGCTTCTCTCAGTTCCGATTGCAG<br>GCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTA<br>ATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGG<br>GCCTTGACACACCGCCCGTCACACCACGAGAGTTTG<br>TAACACCCGAAGTCGGTGAGGTAACCTTTTTGGAGCCA<br>GCCGCTAAGGTGGGATAGATGATTGG | 5 |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|

**[0072]** *E. faecalis* strains frequently harbor a gene encoding the enzyme Gelatinase E or GelE. GelE is a virulence factor of *E. faecalis*. It may contribute to the survival of bacteria in various host tissues, and has been shown enhance biofilm formation *in vitro*.

**[0073]** GelE is a 30-kDa metalloprotease secreted from *E. faecalis* strains and is capable of hydrolyzing gelatin, collagen, casein, hemoglobin, and other peptides. An illustrative sequence of GelE is shown in Table 4, below. As will be understood by those of skill in the art, many different variants of GelE are known, for example as shown in Uniprot Accession No. Q833V7.

**Table 4:** GelE amino acid sequence

| Name | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | SEQ ID No: |
|------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| GelE | MMKGNKILYILGTGIFVGSSCLFSSLFVAAEEQVYSESEVSTVLS<br>KLEKEAISEAAAEQYTVVDRKEDAWGMKHLKLEKQTEGVTVD<br>DNVIIHLDRNGAVTSVTGNPVDQVVKIQSVDAIGEEGVKKIIASD<br>NPETKDLVFLAIDKRVNNEGQLFYKVRVTSSPTGDPVSLVYKVN<br>ATDGTIMEKQDLTEHVGSEVTLKNSFQVAFNVPVEKSNTGIALH<br>GTDNTGVYHAVVDGKNNYSIIQAPSLVALNQNAVDAYTHGKFV<br>KTYIEDHFQRHSIDDRGMPILSVVDEQHPDAYDNAFWDGKAM<br>RYGETSTPTGKTYASSLDVVGHEMTHGVTEHTAGLEYLGQSG<br>ALNESYSDLMGYIISGASNPEIGADTQSVDRKTGIRNLQTPSKH<br>GQPETMAQYDDRARYKGTPTYDQGGVHYNSGIINRIGYTIQNL<br>GIEKAQTIFYSSLVNYLTPKAQFSDARDAMLAAAKVQYGDEAAS<br>VVSAAFNSAGIGAKEDIQVNQPSESVLVNE | 6          |

**[0074]** *C. perfringens* and Collagenase A (ColA)

**[0075]** In some embodiments, the pathogenic bacterial strain is *C. perfringens*. *C. perfringens* is a spore-forming gram-positive bacterium that is found in many environmental sources as well as in the intestines of humans and animals. *C. perfringens* may be identified by its 16S RNA sequence (see Table 5, below). For example, in some embodiments, a strain of *C. perfringens* has a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of SEQ ID NO: 7. In some embodiments, a strain of *C. perfringens* has a 16S RNA sequence that is at least 97% identical to the sequence of SEQ ID NO: 7. In some embodiments, a *C. perfringens* strain is isolated from a human fecal sample. In some embodiments, a *C. perfringens* strain is an engineered strain, such as a non-toxigenic *C. perfringens* strain engineered to express or overexpress ColA.

**Table 5:** Sequence encoding *C. perfringens* 16S RNA

| Species               | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | SEQ ID NO: |
|-----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <i>C. perfringens</i> | TAAATTGAGAGTTTGATCCTGGCTCAGGATGAACGCTG<br>GCGGCGTGCTTAACACATGCAAGTCGAGCGATGAAGT<br>TTCCTTCGGGAAACGGATTAGCGGCGGACGGGTGAGT<br>AACACGTGGGTAACCTGCCTCATAGAGTGGAATAGCC<br>TTCCGAAAGGAAGATTAATACCGCATAACGTTGAAAGA<br>TGGCATCATCATTCAACCAAAGGAGCAATCCGCTATGA<br>GATGGACCCGCGGCGCATTAGCTAGTTGGTGGGGTAA<br>CGGCCTACCAAGGCGACGATGCGTAGCCGACCTGAGA<br>GGGTGATCGGCCACATTGGGACTGAGACACGGCCCA<br>GACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACA<br>ATGGGGGAAACCCTGATGCAGCAACGCCGCGTGAGTG<br>ATGAAGGTTTTTCGGATCGTAAAGCTCTGTCTTTGGGGA<br>AGATAATGACGGTACCCAAGGAGGAAGCCACGGCTAA<br>CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGA<br>GCGTTATCCGGATTTACTGGGCGTAAAGGGAGCGTAG<br>GCGGATGATTAAGTGGGATGTGAAATACCCGGGCTCA<br>ACTTGGGTGCTGCATTCCAAACTGGTTATCTAGAGTGC<br>AGGAGAGGAGAGTGGAATTCCTAGTGTAGCGGTGAAA<br>TGCGTAGAGATTAGGAAGAACACCAAGTGGCGAAGGCG<br>ACTCTCTGGACTGTAAGTACGCTGAGGCTCGAAAGC<br>GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA<br>CGCCGTAAACGATGAATACTAGGTGTGGGGGTTTTCAA<br>CACCTCCGTGCCGCCGCTAACGCATTAAGTATTCCGC<br>CTGGGGAGTACGGTCGCAAGATTAAACTCAAAGGAA<br>TTGACGGGGACCCGCACAAGTAGCGGAGCATGTGGTT<br>TAATTCGAAGCAACGCGCAAGAACCTTACCTACACTTGA<br>CATCCCTTGCACTTACTCTTAATCGAGGAAATCCCTTCG<br>GGGACAAGGTGACAGGTGGTGCATGGTTGTCGTCAGC<br>TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAG<br>CGCAACCCCTTGTCGTTAGTTACTACCATTAAGTTGAGG<br>ACTCTAGCGAGACTGCCTGGGTAAACCAGGAGGAAGG<br>TGGGGATGACGTCAAATCATCATGCCCTTATGTGTAG<br>GGCTACACACGTGCTACAATGGCTGGTACAGAGAGAT<br>GCAATACCGCGAGGTGGAGCCAACTTAAAAACCAGT<br>CTCAGTTCGGATTGTAGGCTGAAACTCGCCTACATGAA<br>GCTGGAGTTACTAGTAATCGCGAATCAGAATGTCGCG<br>GTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTC<br>ACACCATGAGAGTTGGCAATACCCGAAGTCCGTGAGC<br>TAACCGCAAGGAGGCAGCGGCCGAAGGTAGGGTCAG<br>CGATTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAG<br>AACCTGCGGCTGGATCACCTCCTTT | 7          |

**[0076]** *C. perfringens* strains typically harbor a gene encoding the enzyme Collagenase A or ColA. ColA is a toxin that degrades collagen. ColA plays a role in the virulence of *C. perfringens*, by spreading toxins in cells to host tissue. ColA secretion can also be triggered by pro-inflammatory cytokines during a normal immune response, which can lead to tissue damage. ColA is closely related to, and has similar activity to Collagenase H, an enzyme produced by *C. histolyticum*. Specifically, ColA and ColH both digest collagen, have a high degree of homology in the catalytic domain, and have structural similarity (based on 3D *in silico* modeling).

**[0077]** An illustrative sequence of ColA and ColH is shown in Table 6, below. As will be understood by those of skill in the art, many different variants of these enzymes are known, for example as shown in Uniprot Accession Nos. Q46173 and Q46085.

**Table 6:** ColA and ColH amino acid sequence

| Name | Sequence                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | SEQ ID No: |
|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| ColA | MKKNLKRGETKLKLVVERWSATFTLA AFILFNSSFKVLAADKKV<br>ENSNNQGITREINADQISKTELNNEVATDNNRPLGPSIAPSRAR<br>NNKIYTFDELNRMNYS DLVELIKTISYENVPDLNFNDG SYTFFS<br>NRDRVQAIYGLSDSGRTYTADDDKGIPTLVEFLRAGYYLGFYN<br>KQLSYLNTPLKNECLPAMKAIQYNSNFRLGTKAQDGVVEALG<br>RLIGNASADPEVINNCIYVLSDFKDNIDKYGSNYSKGNVFNLM<br>KGIDYYTNSVIYNTKGYDAKNTEFYNRIDPYMERLESLCTIGDKL<br>NNDNAWLNNALYYTGRMGKFREDPSISQRALERAMKEYPYL<br>SYQYIEAANDLDLNF GGKNSSGNDIDFNKIKADAREKYL PKTYT<br>FDDGKFVVKAGDKVTEEKIKRLYWASKEVKAQFMRVVQNDKA<br>LEEGNPDDILTVVIYNSPEEYKLNRIINGFSTDNGGIYIENIGTFFT<br>YERTPEESIYTLLEELFRHEFTHYLQGRYVVPGMWGQGEFYQE<br>GVLTWYEEGTAEFFAGSTRTDGIKPRKSVTQGLAYDRNNRMS<br>LYGVLHAKYGSWDFYNYGFALS NYMYNNNMGMFNKMTNYIKN<br>NDVSGYKDYIASMSSDYGLNDKYQDYMDSLLNNIDNLDVPLVS<br>DEYVNGHEAKDINEITNDIKEVSNIKDLSSNVEKSQFFTTYDMR<br>GTIVGGRSQGEENDWKDMNSKLN DILKELSKKSWNGYKTVTA<br>YFVNHKVDGNGNYVYDVVFHGMNTDTNTDVHVNKEPKAVIKS<br>DSSVIVEEEINFDGTESKDEDEGEIKAYEWDFGDGEKSNEAKAT<br>HKYNKTGEYEVKLTVTDNNGGINTESKKIKVVEDKPV E VINSE<br>PNNDFEKANQIAKSNMLVKGT LSEEDYSDKY YFDVAKKGNVKI<br>TLNNLNSVGITWTLYKEGDLNNYVLYATGNDGTVLKGEKTLEP<br>GRYYLSVYTYDNQSGTYTVNVKGNL KNEVKETAKDAI KEVENN<br>NDFDKAMKVDSNSKIVGTLSNDDLKDIYSIDIQNPSDLNIVVENL<br>DNIKMNWLLYSADDLSNYVDYANADGNKLSNTCKLNP GKYYLC<br>VYQFENSGTGNYIVNLQNK | 8          |

|      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |   |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
| ColH | MKRKCLSKRLMLAITMATIFTVNSTLPIYAAVDKNNATAAVQNE<br>SKRYTVSYLKTLYNDLVDLLVKTEIENLPDLFQYSSDAKEFYG<br>NKTRMSFIMDEIGRRAPQYTEIDHKGIPTLVEVVVAGFYLGPHN<br>KELNEINKRSFKERVIPSILAIQKNPNFKLGTEVQDKIVSATGLLA<br>GNETAPPEVVNNFTPIQDCIKNIDRYALDDLKSKALFNVLAAPT<br>YDITEYLRATKEKPENTPWYGGKIDGFINELKKLALYGKINDNNS<br>WIIDNGIYHIAPLGKLHSNNKIGIETLTEVMKVYPYLSMQHLQSA<br>DQIKRHYDSKDAEGNKIPLDKFKKEGKEKYCPKTYTFDDGKVII<br>KAGARVEEEKVKRLYWASKEVNSQFFRVYIDKPLEEGNPDDI<br>LTMVIYNSPEEYKLNVLGYDTNNGGMYIEPEGTFFTYEREA<br>QESTYTLLEELFRHEYTHYLQGRYAVPGQWGRTKLYDNDRLTW<br>YEEGGAELFAGSTRTSGILPRKSIVSNIHNTTRNNRYKLSDTVH<br>SKYGASFEFYNYACMFMDYMYNKDMGILNKLNDLAKNNDVDG<br>YDNYIRDLSSNYALNDKYQDHMQERIDNYENLTPFVADDYLV<br>RHAYKNPNEIYSEISEVAKLKDAKSEVKKSQYFSTFTLRGSYTG<br>GASKGKLEDQKAMNKFIDDSLKKLDTSWSGYKTLTAYFTNYK<br>VDSSNRVTYDVVFHGYLPNEGDSKNSLPYGKINGTYKGTEKEK<br>IKFSSEGSFDPDGKIVSYEWDGFGDNKSNEENPEHSYDKVGTY<br>TVKLKVTDDKGESSVSTTTAEIKDLSENKLPVIYMHVPKSGALN<br>QKVVFYGGTYDPDGSAGYQWDFGDSDFSSEQNPSHVYTK<br>KGEYTVTLRVMDSGQMSEKTMKIKITDPVYPIGTEKEPNNSKE<br>TASGPVPGIPVSGTIENTSDQDYFYFDVITPGEVKIDINKLGYG<br>GATWVVYDENNNVSYATDDGQNLSGKFKADKPGRYYIHLYM<br>FNGSYMPYRINIEGSVGR | 9 |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|

**[0078]** Provided herein are compounds that may bind to and/or inhibit BFT, CoIA, and/or GelE. In some embodiments, the compounds of the disclosure bind to BFT, CoIA, and/or GelE with an inhibition constant in the range of about  $10^{-5}$  to about  $10^{-13}$  M, e.g., about  $10^{-5}$  M, about  $10^{-6}$  M, about  $10^{-7}$  M, about  $10^{-8}$  M, about  $10^{-9}$  M, about  $10^{-10}$  M, about  $10^{-11}$  M, about  $10^{-12}$  M, or about  $10^{-13}$  M, including all ranges and values therebetween. In some embodiments, the BFT comprises the amino acid sequence of any one of SEQ ID NO: 2-4. In some embodiments, the BFT comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of any one of SEQ ID NO: 2-4. In some embodiments, the small molecules bind to and/or inhibit at least one of BFT1, BFT2, and BFT3. In some embodiments, the small molecules bind to and/or inhibit BFT1 and BFT2. In some embodiments, the small molecules bind to and/or inhibit BFT1 and BFT3. In some embodiments, the small molecules bind to and/or inhibit BFT2 and BFT3. In some embodiments, the small molecules bind to and/or inhibit BFT1, BFT2, and BFT3.

**[0079]** In some embodiments, the GeIE comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the GeIE comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the sequence of SEQ ID NO: 6. In some embodiments, the small molecules bind to and/or inhibit GeIE.

**[0080]** In some embodiments, the ColA comprises the amino acid sequence of SEQ ID NO: 8. In some embodiments, the ColA comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 8. In some embodiments, the small molecules bind to and/or inhibit ColA. In some embodiments, the small molecules bind to and/or inhibit ColA.

**[0081]** In some embodiments, the small molecules bind and/or inhibit one, two or all three of BFT, GeIE, and ColA. For example, in some embodiments, small molecules bind and/or inhibit only BFT. In some embodiments, the small molecules bind and/or inhibit only GeIE. In some embodiments, the small molecules bind and/or inhibit only ColA. In some embodiments, the small molecules bind and/or inhibit BFT and GeIE. In some embodiments, the small molecules bind and/or inhibit BFT and ColA. In some embodiments, the small molecules bind and/or inhibit ColA and GeIE. In some embodiments, the small molecules bind and/or inhibit BFT, GeIE, and ColA. In some embodiments, a small molecule binds to each of ColA, GeIE, and BFT with similar affinity. In some embodiments, a small molecule binds to each of ColA, GeIE, and BFT with different affinity. In some embodiments, a small molecule inhibits the activity of each of ColA, GeIE, and BFT to a different extent. In some embodiments, a small molecule inhibits the activity of each of ColA, GeIE, and BFT to an approximately equal extent.

**[0082]** The small molecules of the present disclosure may bind to and/or inhibit BFT, ColA and/or GeIE *in vitro*, or *in vivo*. In some embodiments, the small molecules bind to and/or inhibit BFT, ColA and/or GeIE that is bound to a cell membrane. In some embodiments, the small molecules bind to and/or inhibit secreted BFT, ColA and/or GeIE. In some embodiments, the small molecules bind to and/or inhibit intracellular BFT, ColA and/or GeIE.

**[0083]** In some embodiments, the small molecules of the present disclosure decrease BFT, ColA, and/or GeIE activity by at least about 5%, at least about 10%, at least about

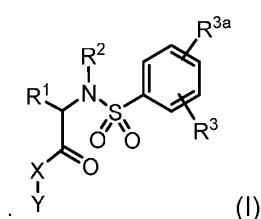
15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%. In some embodiments, the small molecule inhibitors decrease BFT, ColA, and/or GelE activity by about 5% to about 25%, about 25% to about 50%, about 50% to about 75%, or about 75% to 100%. In some embodiments, the small molecule inhibitors decrease BFT, ColA, and/or GelE activity by about 95% to 100%, e.g., a decrease in activity of 95%, 96%, 97%, 98%, 99%, or 100%.

**[0084]** In some embodiments, the small molecules of the present disclosure diminish the pathogenic effects of a strain of *B. fragilis* (ETBF) expressing a BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens* expressing ColA. In some embodiments, the small molecules of the present disclosure substantially eliminate the pathogenic effects of a strain of *B. fragilis* (ETBF) expressing a BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens*. In some embodiments, the small molecules of the present disclosure completely eliminate the pathogenic effects of a strain of *B. fragilis* (ETBF) expressing a BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens*.

**[0085]** In some embodiments, the inhibitor binds to and inhibits the activity of a BFT. In some embodiments, the inhibitor reduces the ability of a BFT to release E-cadherin from a cell. For example, an inhibitor may reduce E-cadherin release by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%. In some embodiments, the inhibitor reduces the ability of a BFT to cause secretion of IL-8 from a cell. For example, an inhibitor may decrease BFT-mediated IL-8 secretion by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95%.

**[0086]** In some embodiments, the compound inhibits BFT, CoIA, and/or GeIE by competitive inhibition. In some embodiments, the inhibitor inhibits BFT, CoIA, and/or GeIE by non-competitive inhibition. In some embodiments, the inhibitor inhibits BFT, CoIA, and GeIE by uncompetitive inhibition. In some embodiments, the inhibitor inhibits BFT, CoIA, and/or GeIE by mixed inhibition (e.g., allosteric inhibition). The inhibition may be reversible, or may be irreversible.

**[0087]** In some embodiments, the present disclosure provides a compound having the structure of Formula I:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-5} \text{ alkyl})-$ ,  $-\text{N}(\text{CH}_2\text{CF}_3)-$ , or  $-\text{O}-$ ;

Y is H,  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{OCH}_2\text{CH}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{CH}_3)$ ,  $-\text{NH}(\text{CH}_2\text{CH}_3)$ , or  $-\text{NH}(\text{CH}_2\text{CF}_3)$ ;

wherein:

when X is  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-5} \text{ alkyl})-$ , or  $-\text{N}(\text{CH}_2\text{CF}_3)-$ , Y is  $-\text{OH}$ ,  $-\text{OCH}_3$ ,  $-\text{OCH}_2\text{CH}_3$ ,  $-\text{NH}_2$ ,  $-\text{NH}(\text{CH}_3)$ ,  $-\text{NH}(\text{CH}_2\text{CH}_3)$ , or  $-\text{NH}(\text{CH}_2\text{CF}_3)$ ; and

when X is  $-\text{O}-$ , Y is H;

$\text{R}^1$  is alkyl, aryl,  $-\text{alkylene-OH}$ ,  $-\text{alkylene-NH}_2$ ,  $-\text{alkylene-C(=O)NH}_2$ , heteroaralkyl, aralkyl,  $-\text{alkylene-S-alkyl}$ ,  $-\text{alkylene-S-haloalkyl}$ ,  $-\text{alkylene-S-aralkyl}$ , or  $-\text{alkylene-S-heteroaralkyl}$ ; wherein  $\text{R}^1$  is optionally substituted with one or more groups selected from  $-\text{OH}$ ,  $-\text{OMe}$ , halogen,  $-\text{CHF}_2$ ,  $-\text{CH}_2\text{F}$ , or  $-\text{CF}_3$ ;

$\text{R}^2$  is H,  $-(\text{CH}_2)_n\text{-aryl}$ ,  $-\text{CH}_2\text{-alkyl}$ ,  $-\text{CH}(\text{Me})\text{-alkyl}$ ,  $-\text{CH}_2\text{-heterocyclyl}$ ,  $-(\text{CH}_2)_n\text{-heteroaryl}$ , or  $-\text{CH}_2\text{-haloalkyl}$ ; and

$\text{R}^3$  is  $-\text{OH}$ , alkoxy,  $-\text{O-alkylene-NR}^5\text{R}^6$ , alkyl,  $-\text{S-alkyl}$ ,  $-\text{NH-alkyl}$ ,  $-\text{N}(\text{CH}_3)\text{-alkyl}$ ,  $-\text{N(H)aralkyl}$ ,  $-\text{N(H)C(O)-alkylene-NR}^5\text{R}^6$ ,  $-\text{O-haloalkyl}$ ,  $-\text{O-aryl}$ ,  $-\text{O-heteroaryl}$ , or  $-\text{O-aralkyl}$ , each of which is optionally substituted;

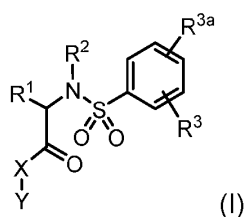
$\text{R}^{3a}$  is H or halogen;

$R^5$  is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl;

$R^6$  is H, alkyl, or aryl; and

$n$  is an integer from 0 to 3.

**[0088]** In some embodiments, the present disclosure provides a compound having the structure of Formula I:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is -NH-, -N(C<sub>1-5</sub> alkyl)-, or -N(CH<sub>2</sub>CF<sub>3</sub>)-, Y is -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>); and

when X is -O-, Y is H;

$R^1$  is alkyl, -alkylene-OH, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, heteroaralkyl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein  $R^1$  is optionally substituted with one or more groups selected from -OH, -OMe, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>;

$R^2$  is H, -CH<sub>2</sub>-aryl, CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -CH<sub>2</sub>-heteroaryl, or CH<sub>2</sub>-haloalkyl; and

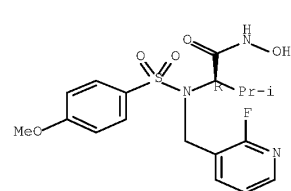
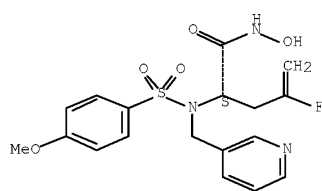
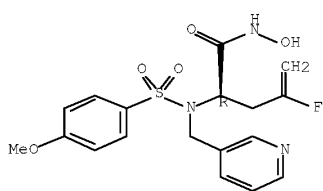
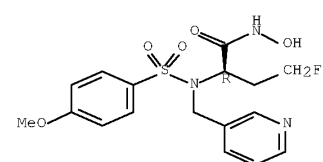
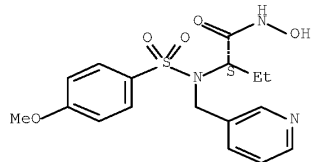
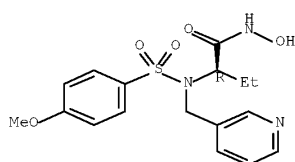
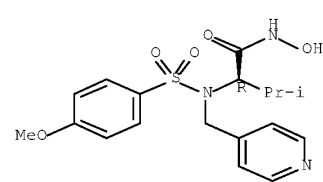
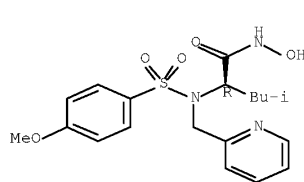
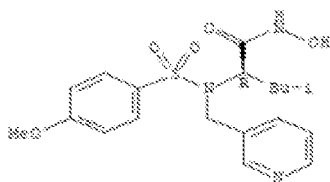
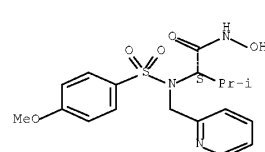
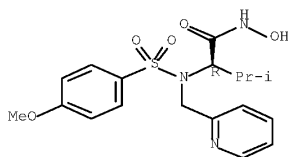
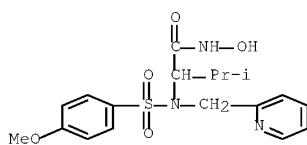
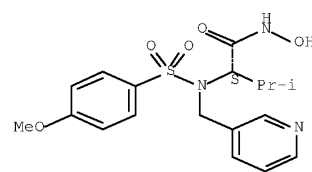
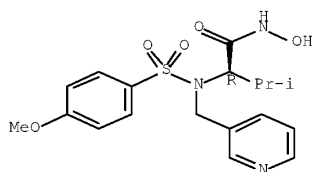
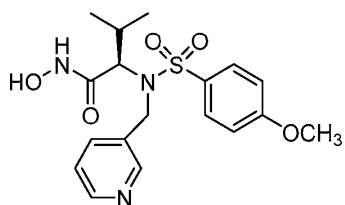
$R^3$  is -OH, alkoxy, -O-alkylene-NR<sup>5</sup>R<sup>6</sup>, alkyl, -S-alkyl, -NH-alkyl, -N(CH<sub>3</sub>)-alkyl, -N(H)aralkyl, -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>, -O-haloalkyl, -O-aryl, -O-heteroaryl, or -O-aralkyl, each of which is optionally substituted;

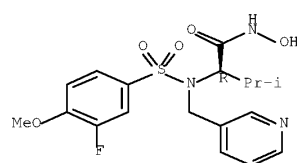
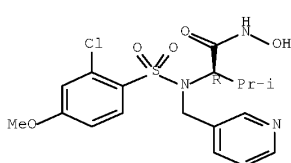
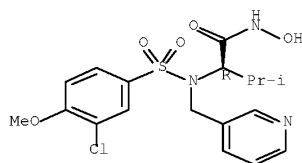
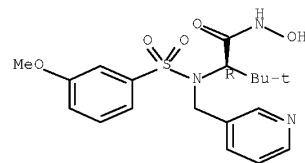
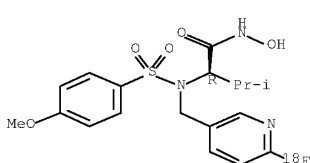
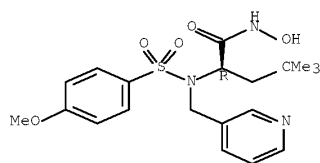
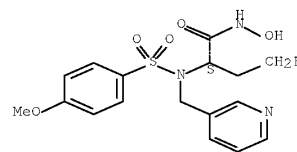
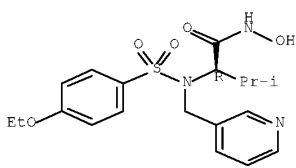
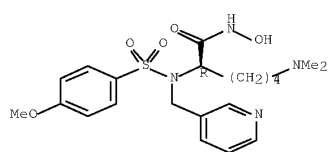
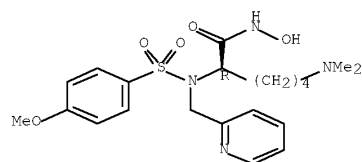
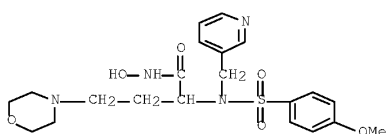
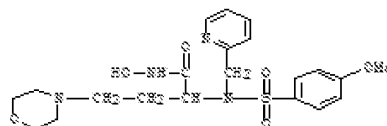
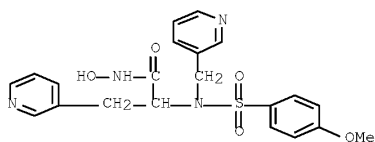
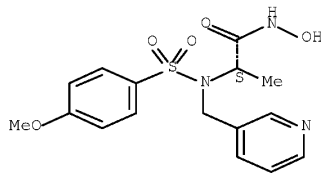
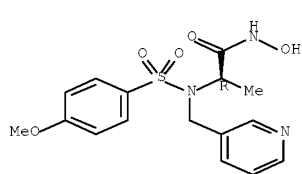
$R^{3a}$  is H or halogen;

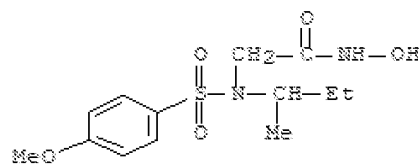
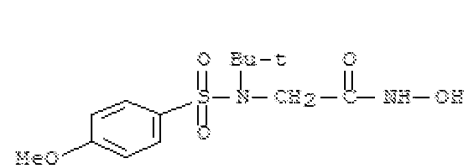
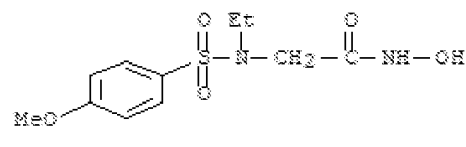
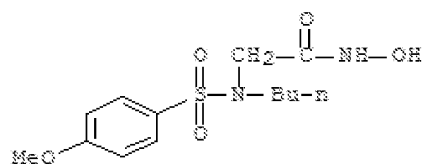
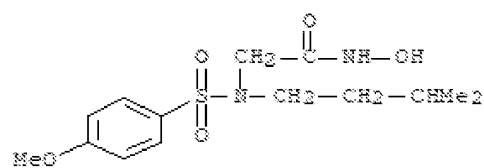
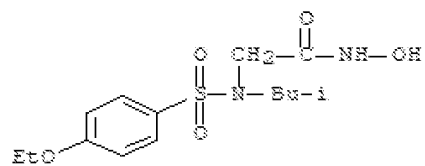
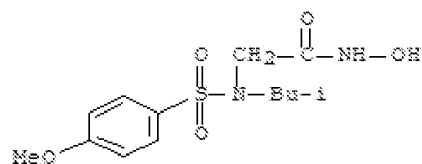
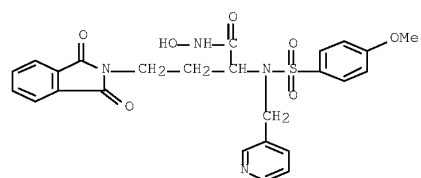
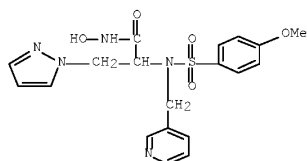
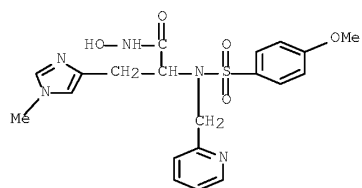
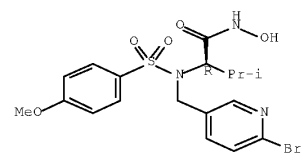
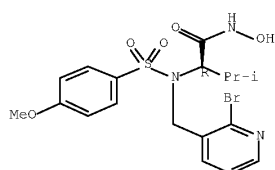
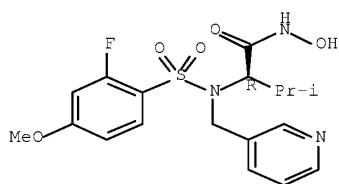
$R^5$  is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl; and

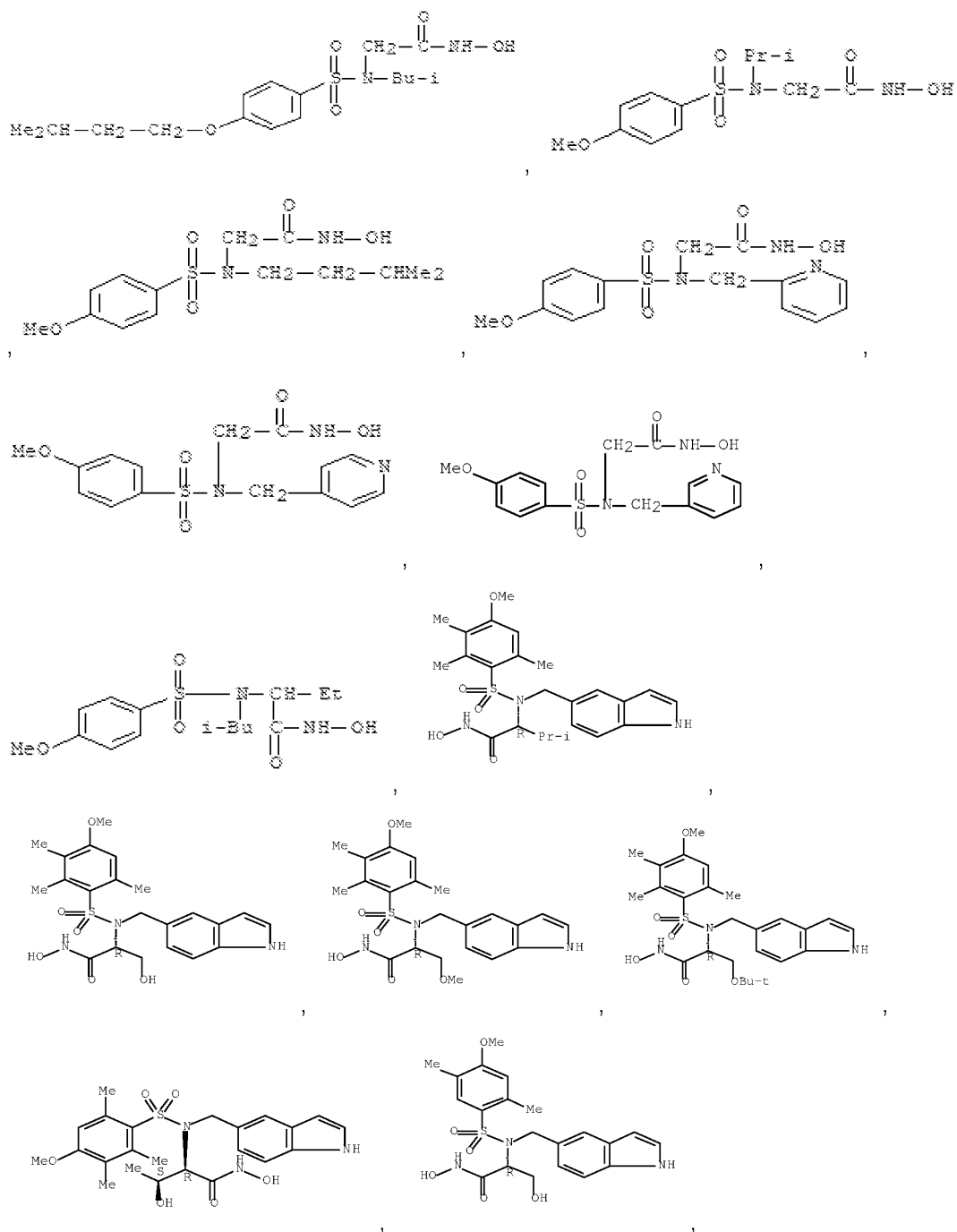
$R^6$  is H, alkyl, or aryl.

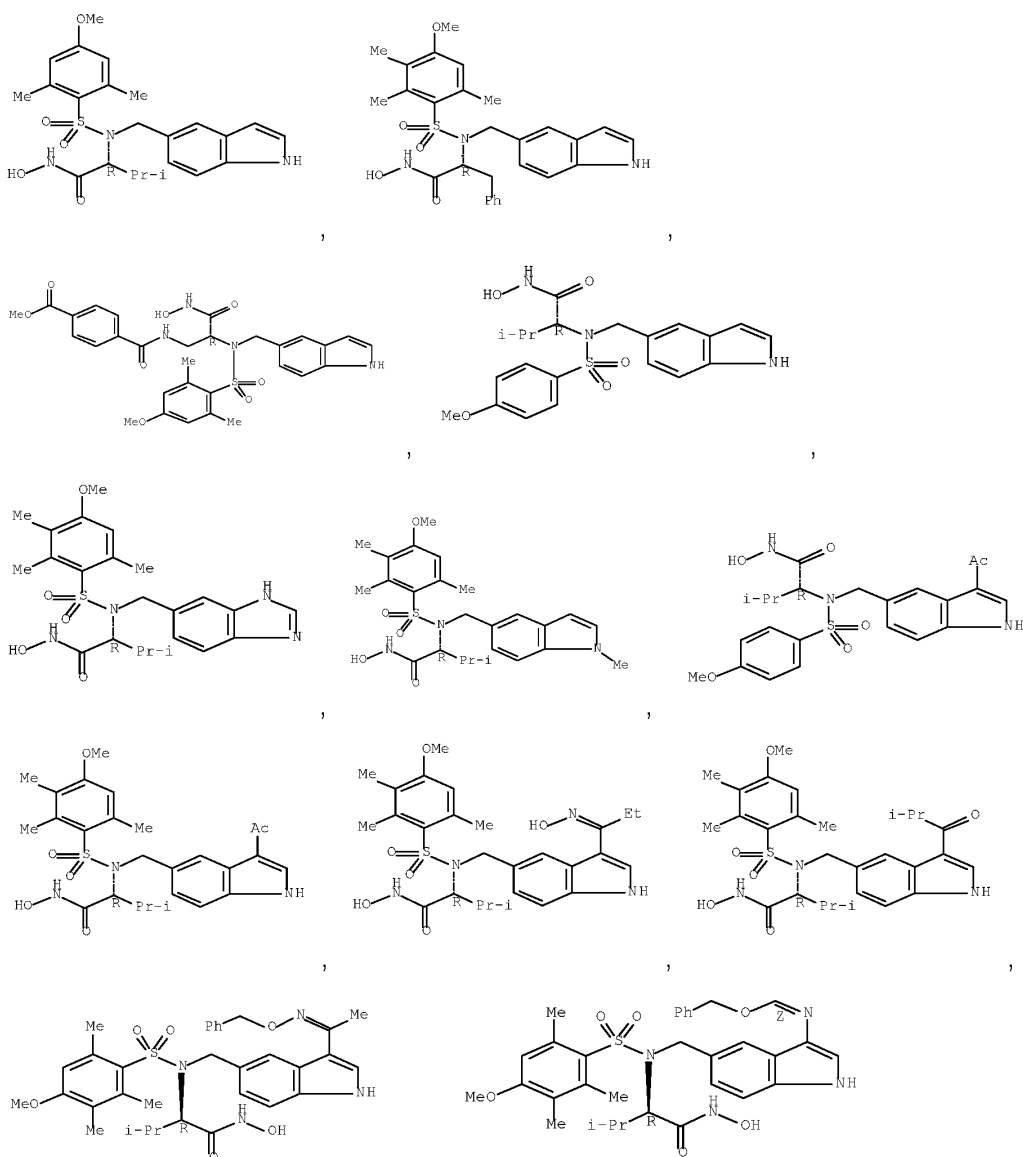
**[0089]** In some embodiments, the compound of the present disclosure is not one or more of the following compounds:









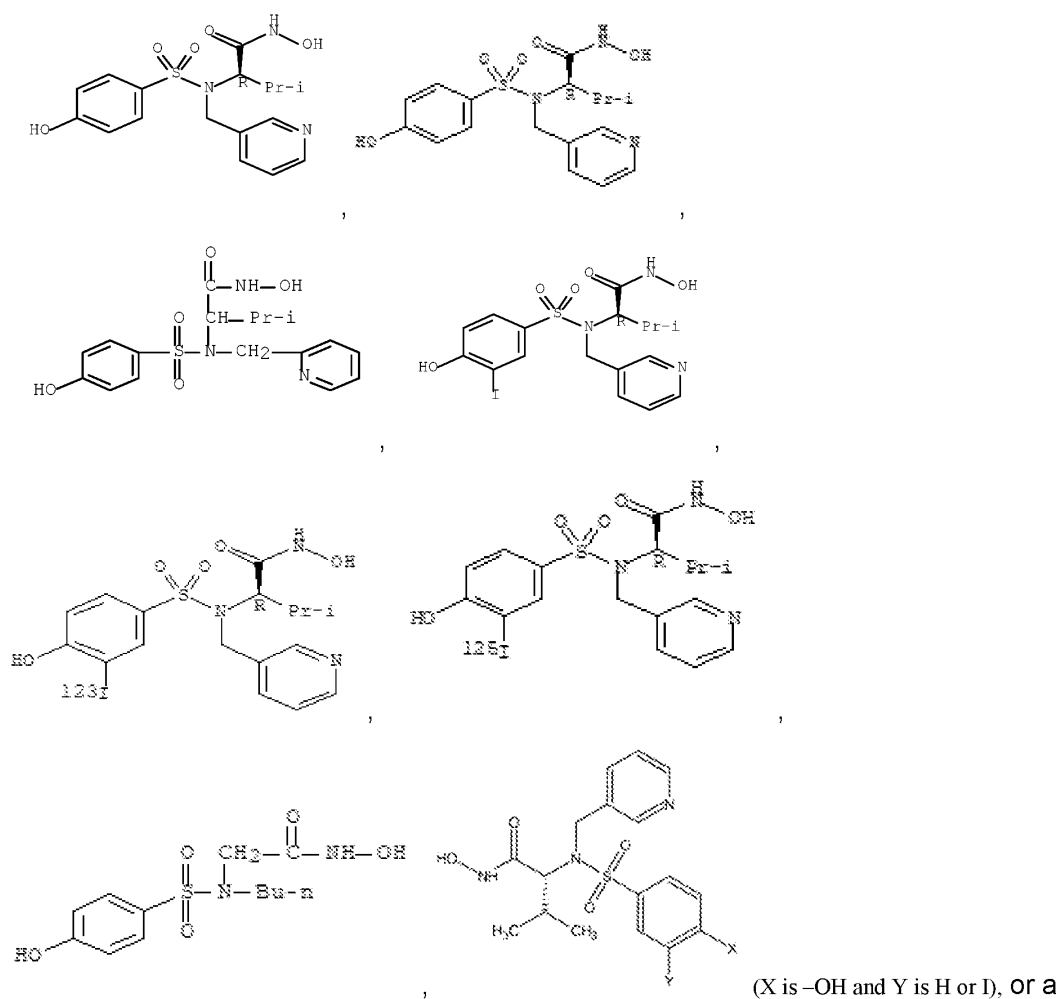


, or

, or a

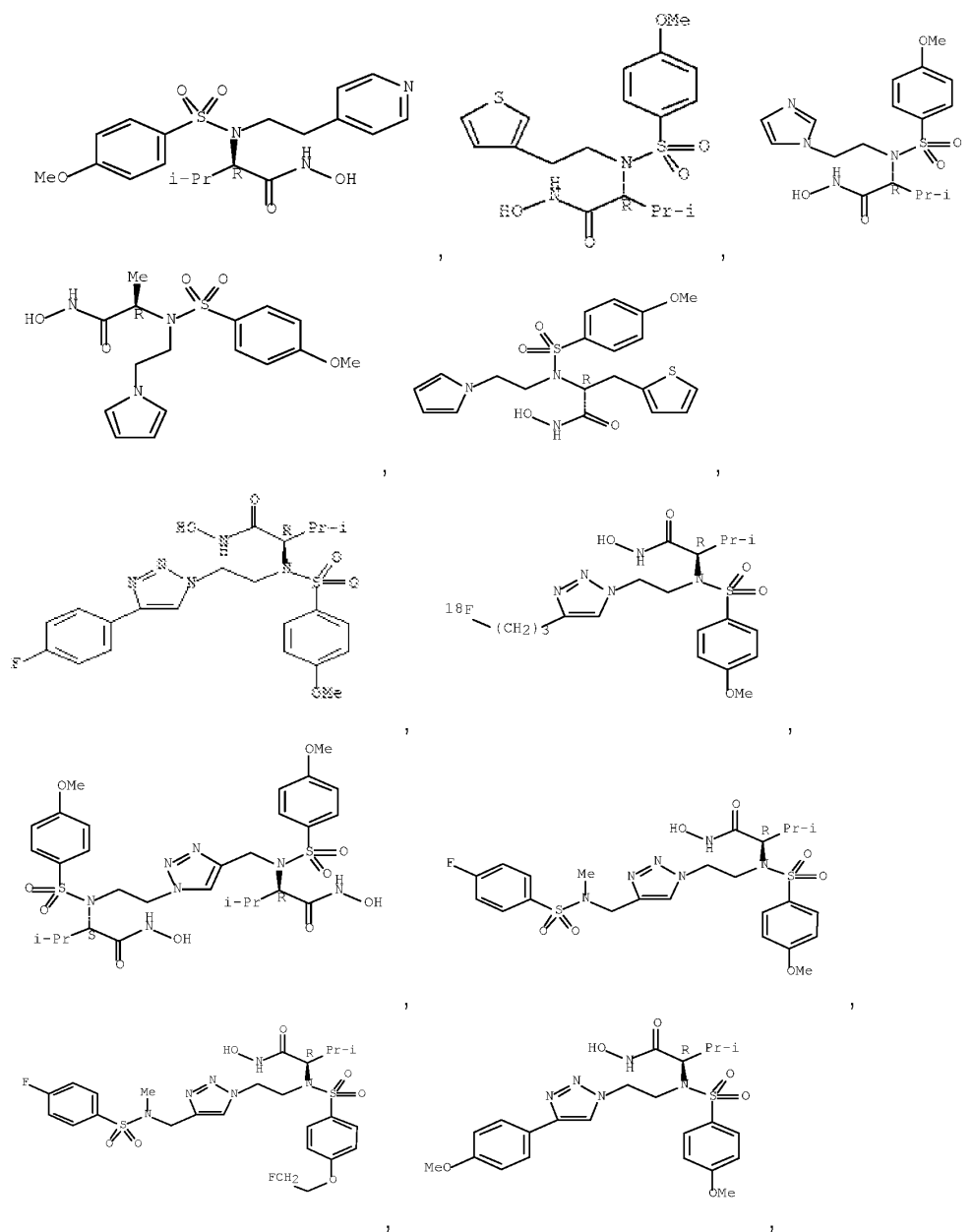
pharmaceutically acceptable salt thereof.

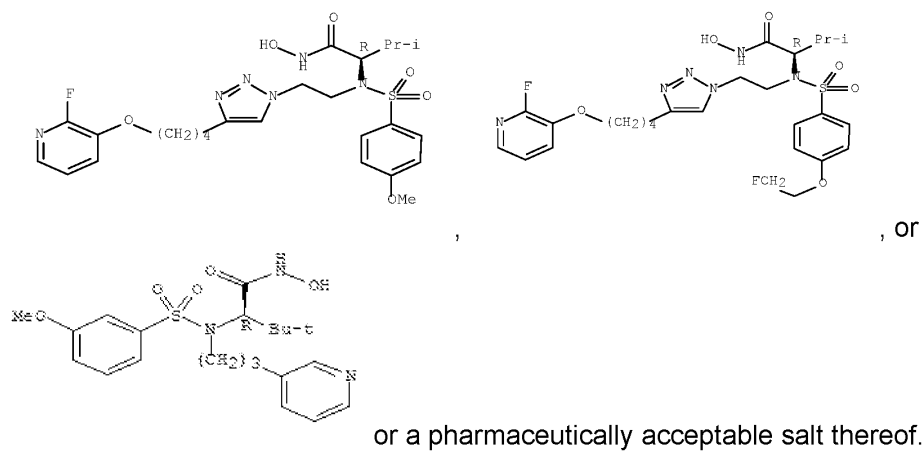
**[0090]** In some embodiments, the compound of the present disclosure is not one or more of the following compounds:



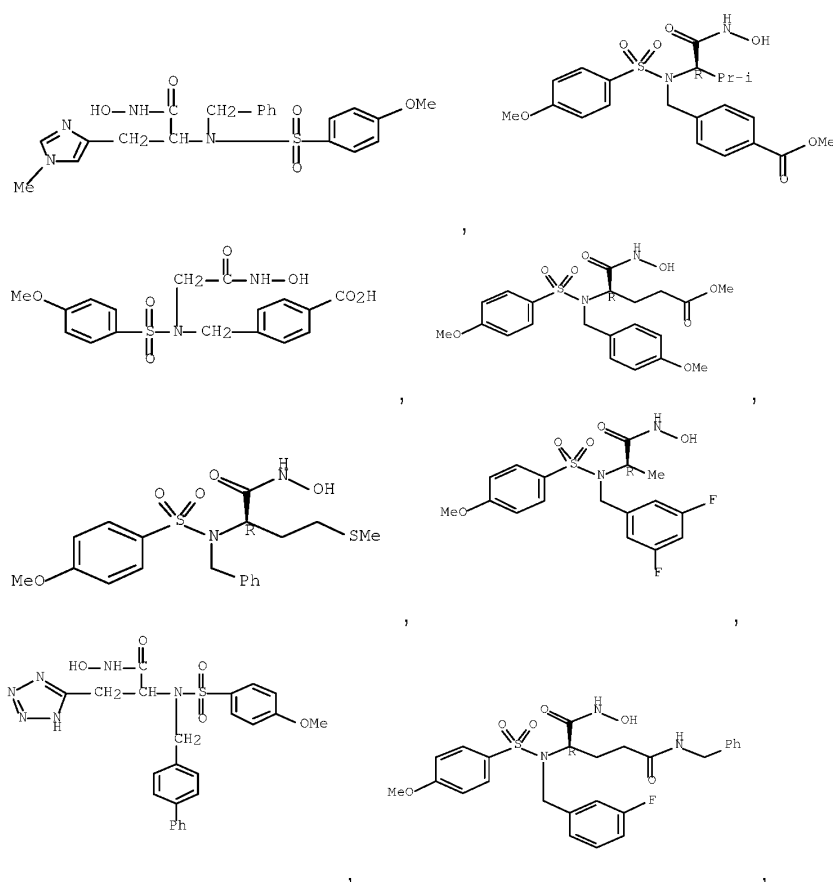
pharmaceutically acceptable salt thereof.

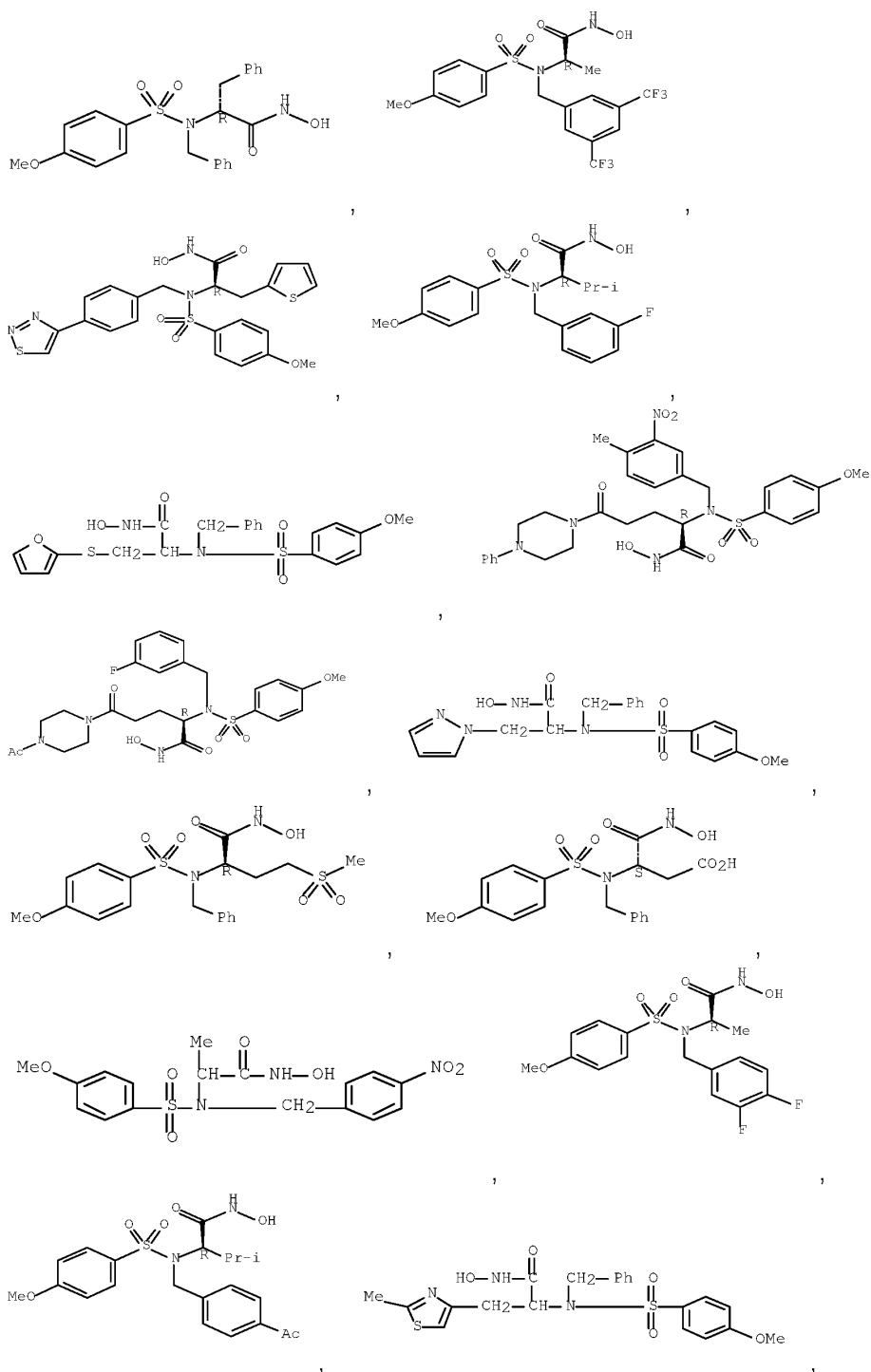
**[0091]** In some embodiments, the compound of the present disclosure is not one or more of the following compounds:

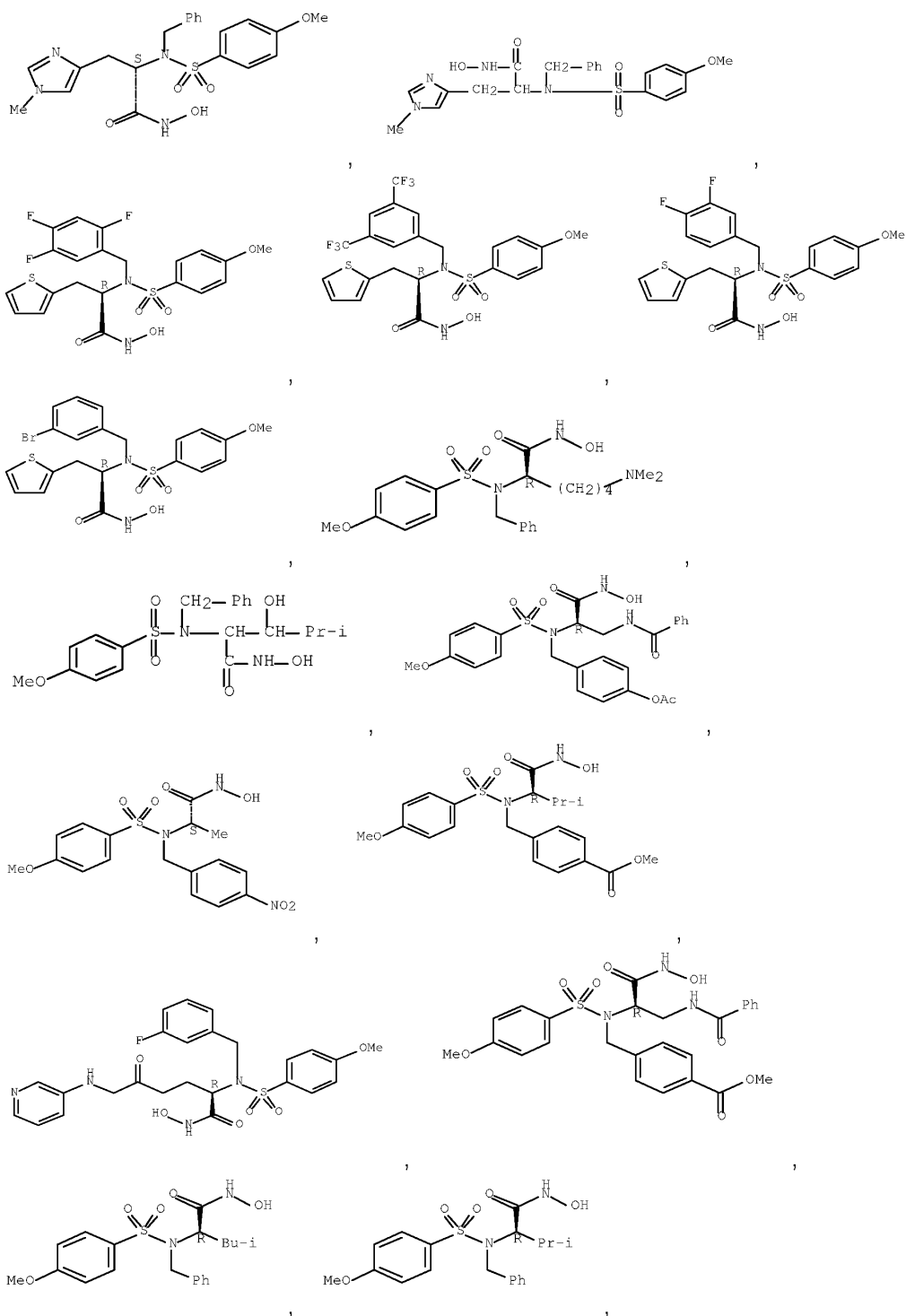


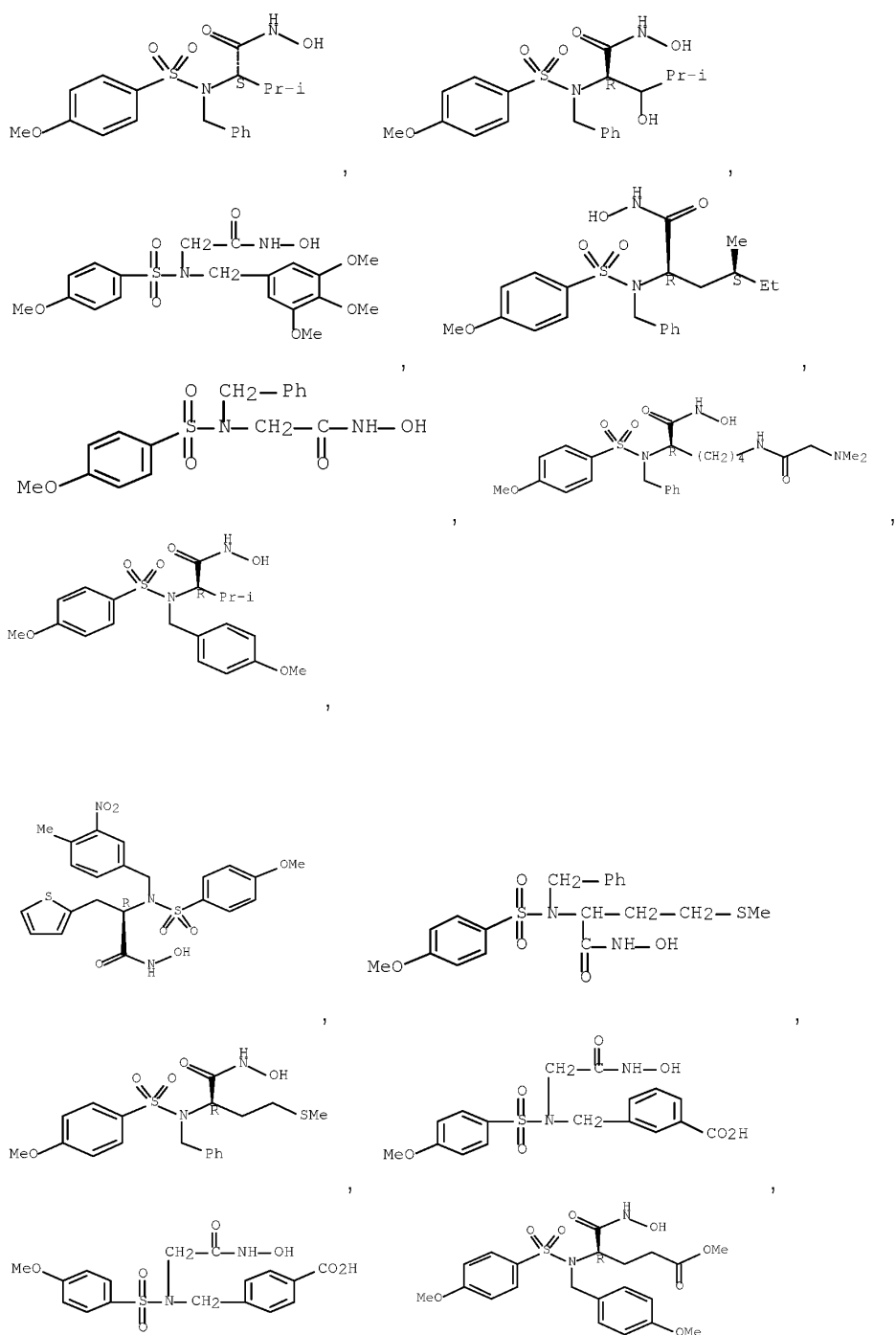


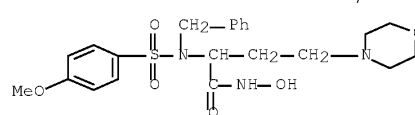
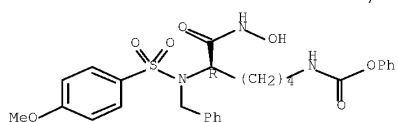
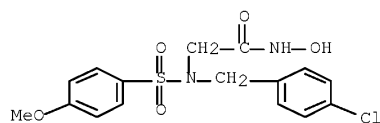
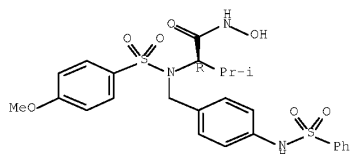
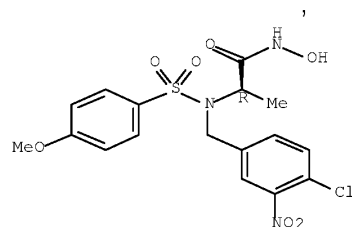
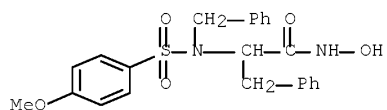
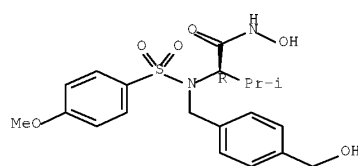
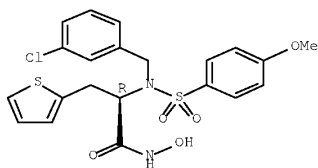
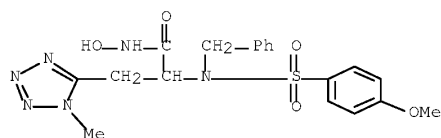
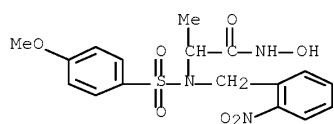
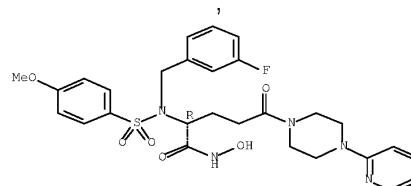
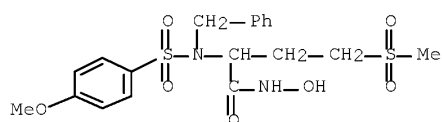
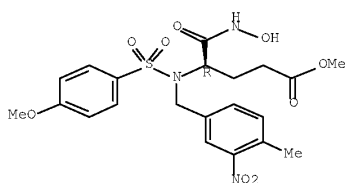
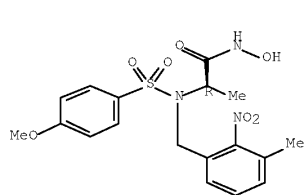
**[0092]** In some embodiments, the compound of the present disclosure is not one or more of the following compounds:

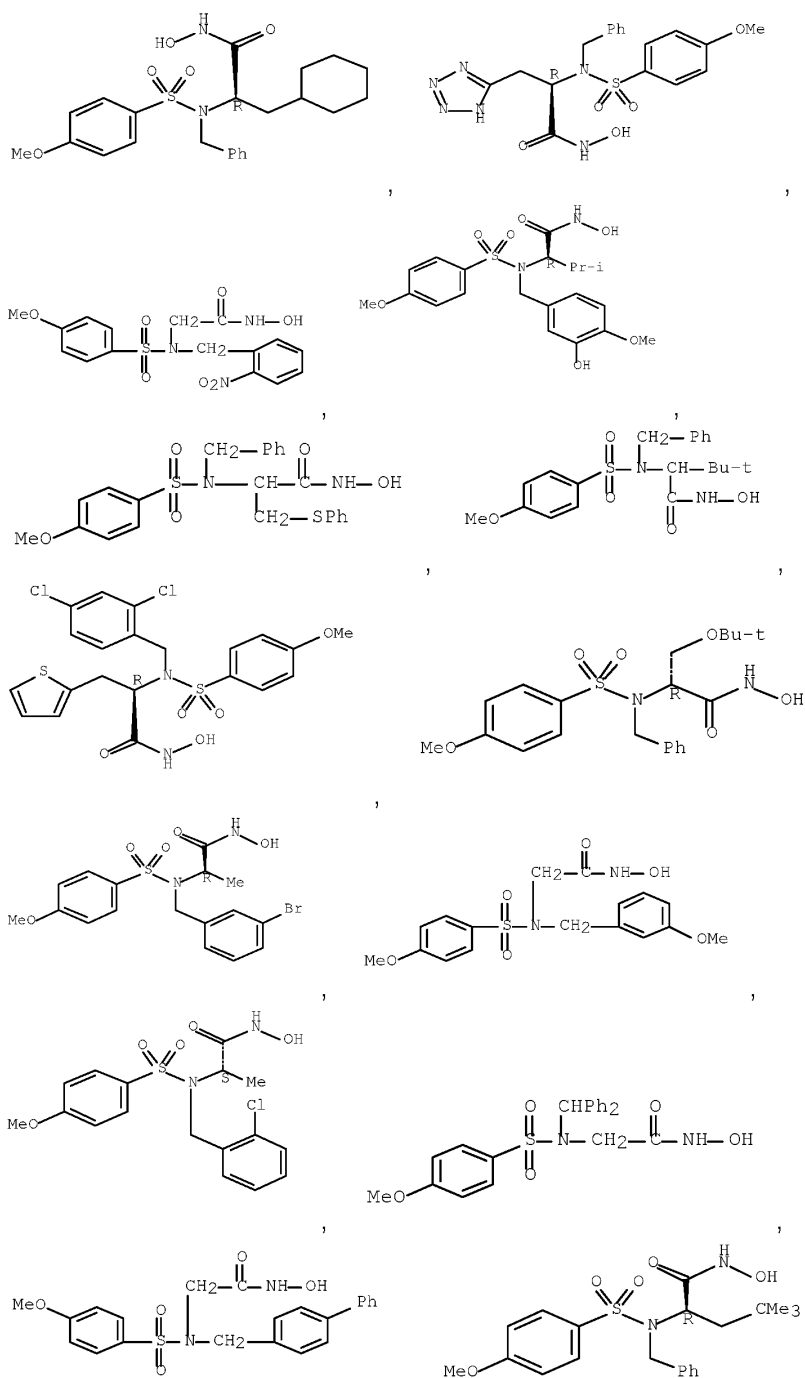


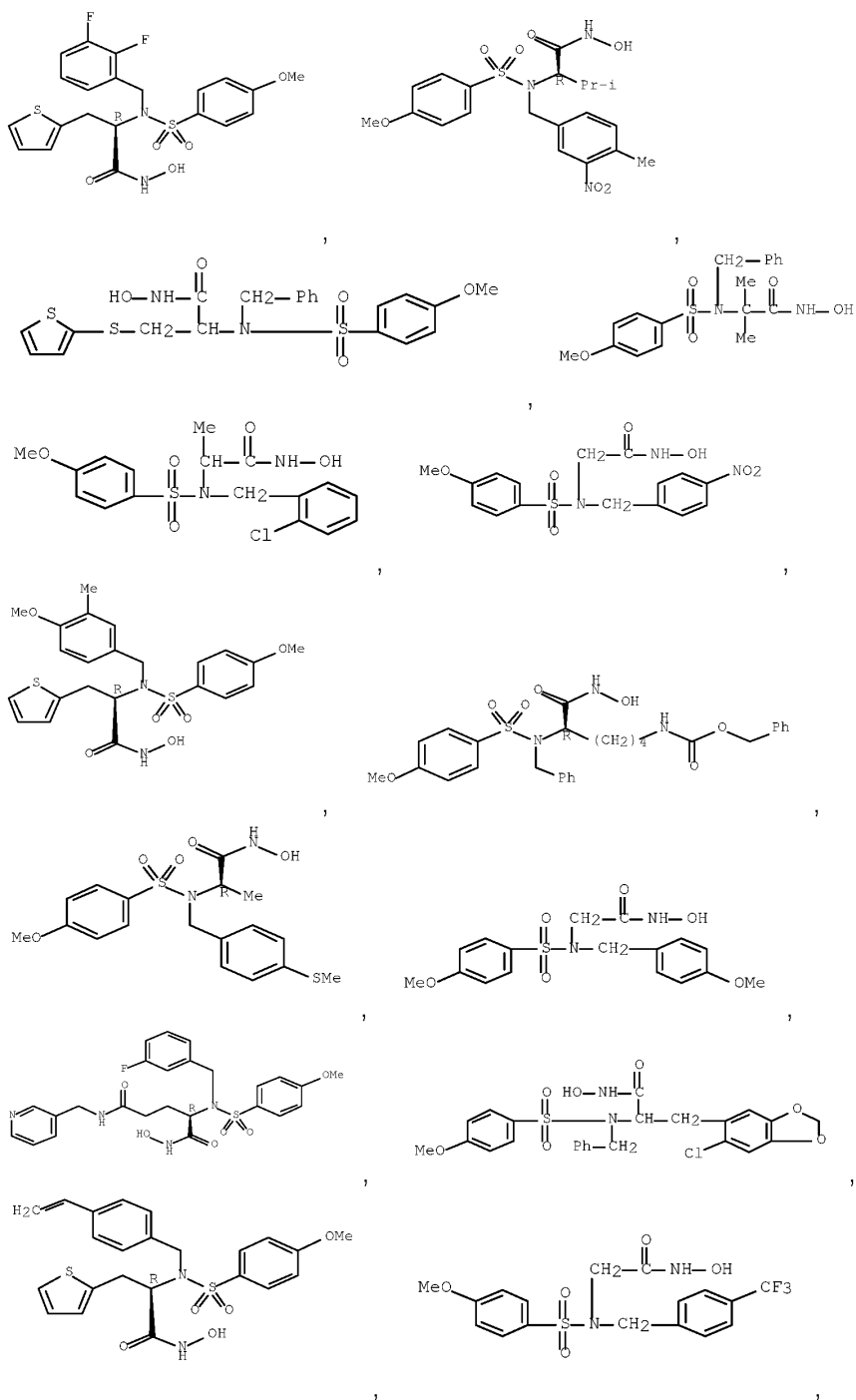


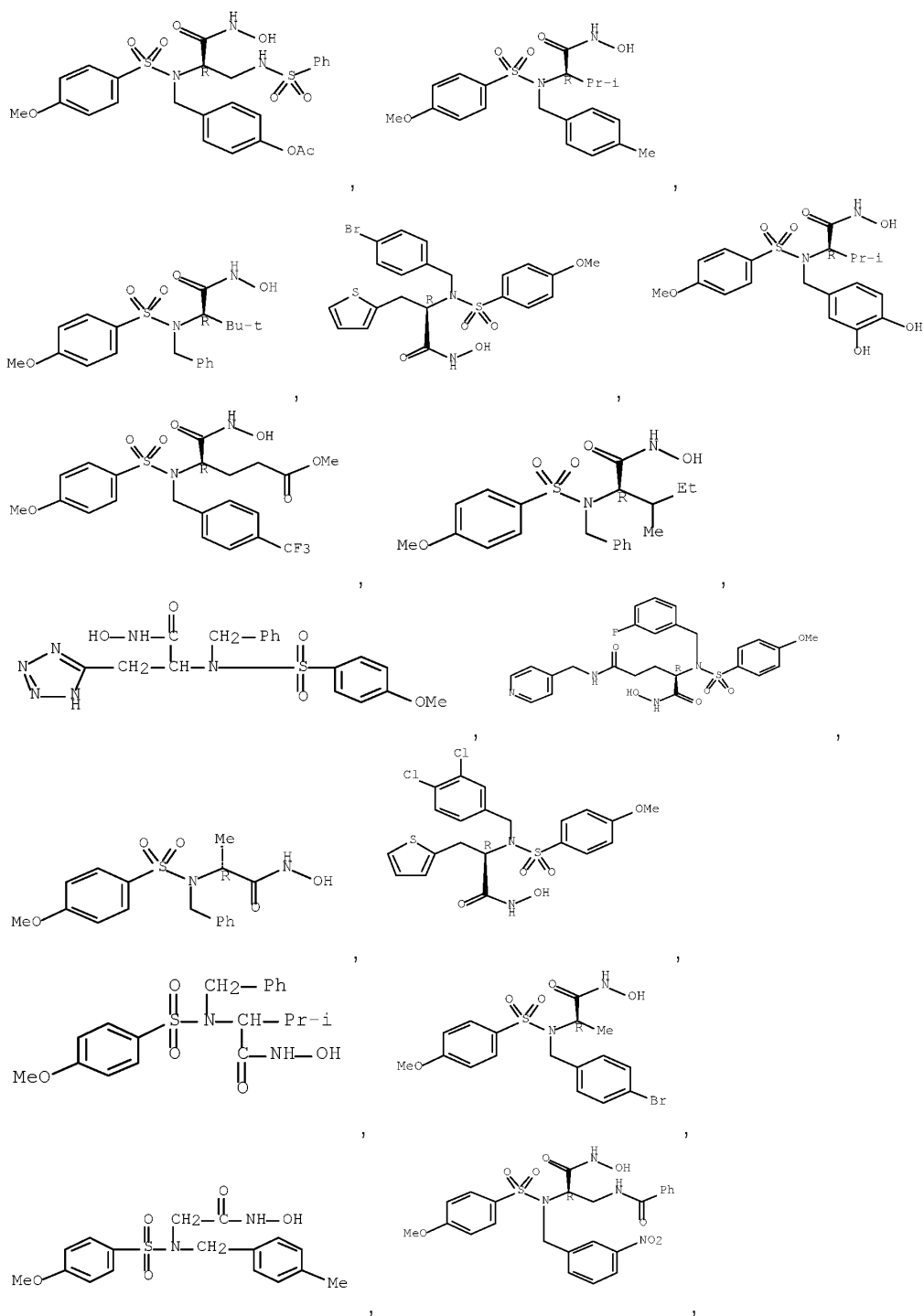


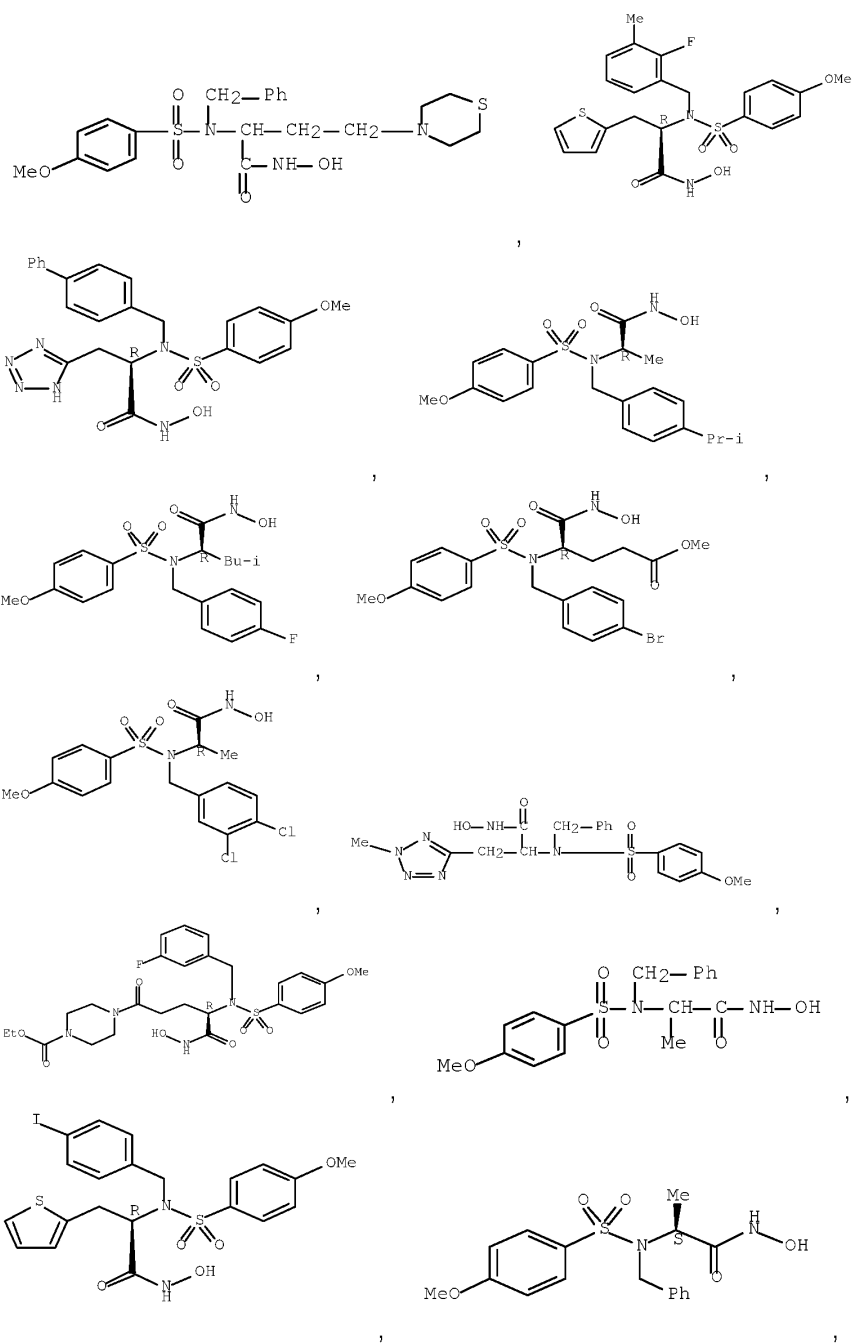


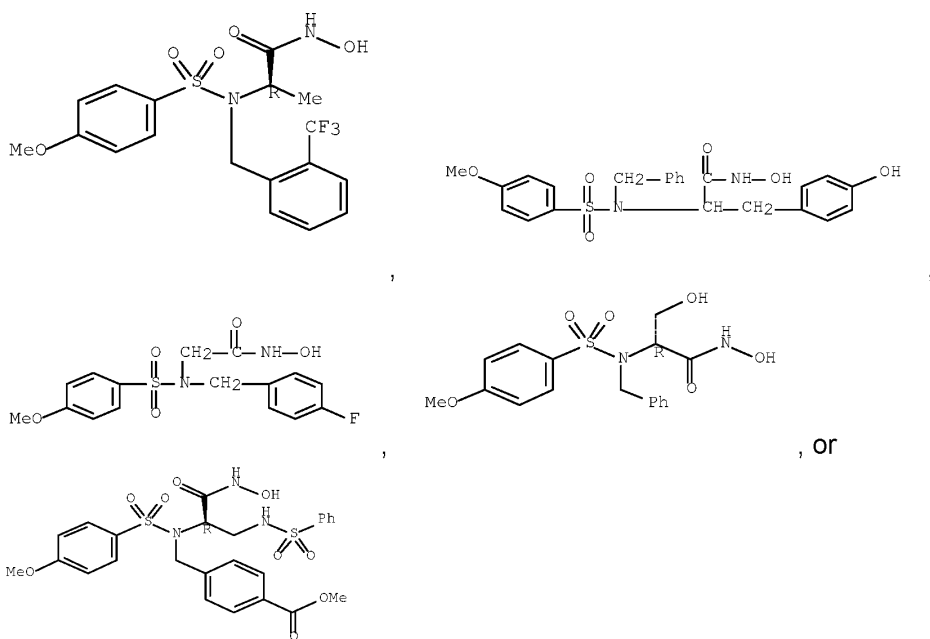






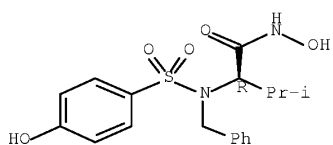






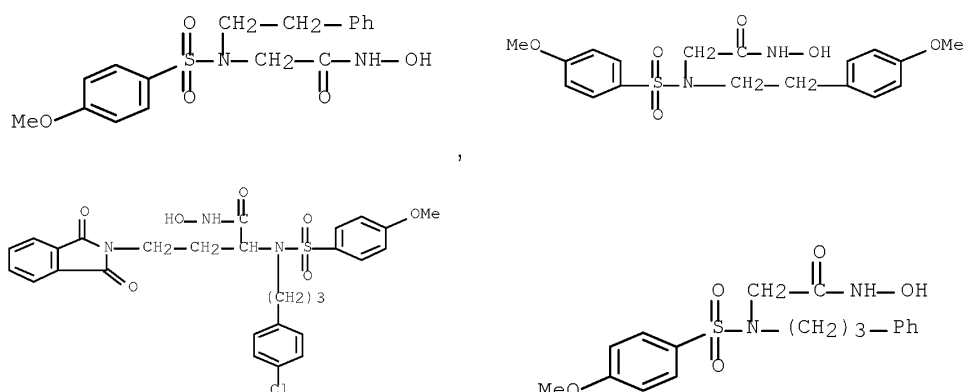
, or a pharmaceutically acceptable salt thereof.

**[0093]** In some embodiments, the compound of the present disclosure is not:



or a pharmaceutically acceptable salt thereof.

**[0094]** In some embodiments, the compound of the present disclosure is not one or more of the following compounds:



, or

, or a

pharmaceutically acceptable salt thereof.

**[0095]** In some embodiments of Formula I, X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$ . In some embodiments, X is  $\text{—NH—}$  or  $\text{—N(C}_{1-5}\text{ alkyl)—}$ . In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl, ethyl, propyl, or isopropyl. In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl or ethyl. In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl. In some embodiments, X is  $\text{—NH—}$ ,  $\text{—N(CH}_3\text{)—}$ ,  $\text{—N(CH}_2\text{CH}_3\text{)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$ . In some embodiments, X is  $\text{—NH—}$ .

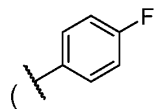
**[0096]** In some embodiments of Formula I, Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ ,  $\text{—NH(C}_{1-5}\text{ alkyl)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ . In some embodiments, Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ , or  $\text{—NH(C}_{1-5}\text{ alkyl)}$ . In some embodiments, Y is  $\text{—OH}$  or  $\text{—OC}_{1-5}\text{ alkyl}$ . In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl, ethyl, propyl, or isopropyl. In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl or ethyl. In some embodiments, the  $\text{C}_{1-5}$  alkyl is methyl. In some embodiments, Y is  $\text{—OH}$ ,  $\text{—OCH}_3$ ,  $\text{—OCH}_2\text{CH}_3$ ,  $\text{—NH}_2$ ,  $\text{—NH(CH}_3\text{)}$ ,  $\text{—NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ . In some embodiments, Y is  $\text{—OH}$ ,  $\text{—OCH}_3$ , or  $\text{—OCH}_2\text{CH}_3$ . In some embodiments, Y is  $\text{—OH}$ .

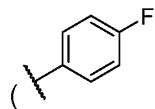
**[0097]** In some embodiments of Formula I, X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$  and Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ ,  $\text{—NH(C}_{1-5}\text{ alkyl)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ . In some embodiments, X is  $\text{—NH—}$  or  $\text{—N(C}_{1-5}\text{ alkyl)—}$  and Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ , or  $\text{—NH(C}_{1-5}\text{ alkyl)}$ . In some embodiments, X is  $\text{—NH—}$  or  $\text{—N(C}_{1-5}\text{ alkyl)—}$  and Y is  $\text{—OH}$  or  $\text{—OC}_{1-5}\text{ alkyl}$ . In some embodiments, X is  $\text{—NH—}$ ,  $\text{—N(CH}_3\text{)—}$ ,  $\text{—N(CH}_2\text{CH}_3\text{)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$  and Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ , or  $\text{—NH(C}_{1-5}\text{ alkyl)}$ . In some embodiments, X is  $\text{—NH}$  and Y is  $\text{—OH}$ ,  $\text{—OC}_{1-5}\text{ alkyl}$ ,  $\text{—NH}_2$ , or  $\text{—NH(C}_{1-5}\text{ alkyl)}$ . In some embodiments, X is  $\text{—NH}$  and Y is  $\text{—OH}$  or  $\text{—OC}_{1-5}\text{ alkyl}$ . In some embodiments, X is  $\text{—NH}$  and Y is  $\text{—OH}$ . In some embodiments,

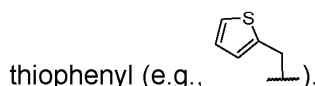
the C<sub>1-5</sub> alkyl is methyl, ethyl, propyl, or isopropyl. In some embodiments, the C<sub>1-5</sub> alkyl is methyl or ethyl. In some embodiments, the C<sub>1-5</sub> alkyl is methyl.

**[0098]** In some embodiments of Formula I, R<sup>1</sup> is H, alkyl, haloalkyl -alkylene-OH, alkylene-O-alkyl, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, -alkylene-S-heteroaralkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is alkyl, haloalkyl -alkylene-OH, alkylene-O-alkyl, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, -alkylene-S-heteroaralkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is H, alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, -alkylene-S-alkyl, -alkylene-S-haloalkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, -alkylene-S-alkyl, -alkylene-S-haloalkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is H, alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-S-alkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-S-alkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is alkyl, -alkylene-OH, alkylene-O-alkyl, heteroaralkyl, aryl, or aralkyl. In some embodiments, R<sup>1</sup> is H or alkyl, aryl, -alkylene-OH, or alkylene-O-alkyl. In some embodiments, R<sup>1</sup> is alkyl, -alkylene-OH, or alkylene-O-alkyl. In some embodiments, R<sup>1</sup> is alkyl, aryl, or heteroaralkyl. In some embodiments, R<sup>1</sup> is alkyl or aryl. In some embodiments, R<sup>1</sup> is alkyl. In some embodiments, the alkyl is a C<sub>1-6</sub> alkyl. In some embodiments, the alkylene is a propylene. In some embodiments, the alkyl is a C<sub>1-6</sub> alkyl. In some embodiments, the alkyl is a C<sub>2-6</sub> alkyl. In some embodiments, the alkyl is ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, isoamyl, or isopentyl. In some embodiments, the alkyl is ethyl, propyl, isopropyl, or isobutyl. In some embodiments, the alkyl is ethyl, propyl, or isobutyl. In some embodiments, alkyl is methyl or ethyl. In some embodiments, the alkyl is ethyl. In some embodiments, alkyl is methyl. In some embodiments, the aryl is a C<sub>6-12</sub> aryl. In some embodiments, the aryl (e.g., a C<sub>6-12</sub> aryl) is phenyl. In some embodiments, the phenyl is substituted with one or more halogen, C<sub>1-5</sub> alkyl, or -O-C<sub>1-5</sub> alkyl. In some embodiments, the phenyl is substituted with one or more halogens. In some embodiments, the phenyl is 3-fluorophenyl, 3-methoxyphenyl, 3-chlorophenyl, 4-fluorophenyl, 3-fluorophenyl, 3-difluoromethylphenyl,

3-thiomethylphenyl, 4-tolyl, or 3-tolyl. In some embodiments, the phenyl is 4-fluorophenyl

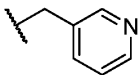


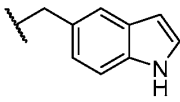
(). In some embodiments, the alkylene is a C<sub>1-5</sub> alkylene. In some embodiments, the alkylene is a C<sub>1-3</sub> alkylene. In some embodiments, the alkylene is a methylene (–CH<sub>2</sub>–) or ethylene (–CH<sub>2</sub>CH<sub>2</sub>–). In some embodiments, the alkylene is an ethylene or propylene (–CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>–). In some embodiments, the alkylene is a methylene. In some embodiments, the alkylene is an ethylene. In some embodiments, the haloalkyl is CF<sub>3</sub>, CHF<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>CF<sub>3</sub>, CH<sub>2</sub>CHF<sub>2</sub>, or CF<sub>2</sub>CF<sub>3</sub>. In some embodiments, the heteroaryl is 2-thiophenyl. In some embodiments, the heteroaryl is an In some embodiments, the heteroaralkyl is –CH<sub>2</sub>–(2-thiophenyl). In some embodiments, the aralkyl is –CH<sub>2</sub>aryl. In some embodiments, the aralkyl is –CH<sub>2</sub>-phenyl, –CH<sub>2</sub>-(4-hydroxyphenyl), –CH<sub>2</sub>-(4-methoxyphenyl), –CH<sub>2</sub>-(4-thiomethylphenyl), –CH<sub>2</sub>-(4-nitrophenyl), –CH<sub>2</sub>-(3-trifluoromethylphenyl), –CH<sub>2</sub>-(4-trifluoromethylphenyl), –CH<sub>2</sub>-(4-difluoromethylphenyl), –CH<sub>2</sub>-(3-fluorophenyl), or –CH<sub>2</sub>-(4-fluorophenyl). In some embodiments, the heteroaralkyl is –CH<sub>2</sub>-heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from N, O, and S. In some embodiments, the heteroaryl is pyridyl, thiophenyl, oxazolyl, or thiazolyl. In some embodiments, the heteroaralkyl is –CH<sub>2</sub>-indolyl, –CH<sub>2</sub>-imidazolyl, –CH<sub>2</sub>-oxazolyl, –CH<sub>2</sub>-thiazolyl, or –CH<sub>2</sub>-thiophenyl. In some embodiments, the heteroaralkyl is –CH<sub>2</sub>-

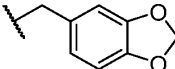
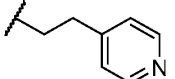


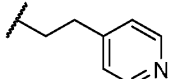
**[0099]** In some embodiments of Formula I, R<sup>2</sup> is H, –(CH<sub>2</sub>)<sub>n</sub>-aryl, –(CH<sub>2</sub>)<sub>n</sub>-alkyl, –CH(Me)-alkyl, –(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or –(CH<sub>2</sub>)<sub>n</sub>-haloalkyl. In some embodiments, R<sup>2</sup> is –(CH<sub>2</sub>)<sub>n</sub>-aryl, –(CH<sub>2</sub>)<sub>n</sub>-alkyl, –(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or –(CH<sub>2</sub>)<sub>n</sub>-haloalkyl. In some embodiments, R<sup>2</sup> is –(CH<sub>2</sub>)<sub>n</sub>-alkyl, –(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or –(CH<sub>2</sub>)<sub>n</sub>-haloalkyl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-aryl, –CH<sub>2</sub>-alkyl, –CH(Me)-alkyl, –CH<sub>2</sub>-heteroaryl, or –CH<sub>2</sub>-haloalkyl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-aryl, –CH<sub>2</sub>-alkyl, –CH<sub>2</sub>-heteroaryl, or –CH<sub>2</sub>-haloalkyl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-aryl, –CH<sub>2</sub>-alkyl, or –CH<sub>2</sub>-heteroaryl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-alkyl or –CH<sub>2</sub>-heteroaryl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-alkyl. In some embodiments, R<sup>2</sup> is –CH<sub>2</sub>-alkyl, wherein the alkyl is optionally substituted with aryl or

heteroaryl. In some embodiments,  $R^2$  is  $-\text{CH}_2\text{-alkyl}$ , wherein the alkyl is optionally substituted with aryl. In some embodiments,  $R^2$  is  $-\text{CH}_2\text{-alkyl}$ , wherein the alkyl is optionally substituted with heteroaryl. In some embodiments, the alkyl is a  $\text{C}_{1-6}$  alkyl. In some embodiments, the alkyl is a  $\text{C}_{2-6}$  alkyl. In some embodiments, the alkyl is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, isoamyl, or isopentyl. In some embodiments, the alkyl is methyl, ethyl, propyl, isopropyl, or isobutyl. In some embodiments, the alkyl is ethyl, propyl, or isobutyl. In some embodiments, alkyl is methyl or ethyl. In some embodiments, the alkyl is not isopropyl. In some embodiments, alkyl is methyl. In some embodiments,  $R^2$  is  $-\text{CH}_2\text{-heteroaryl}$ . In some embodiments, the aryl is a phenyl. In some embodiments, the heteroaryl is a 5- to 14-membered heteroaryl. In some embodiments, the heteroaryl is a 6- to 14-membered heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl. In some embodiments, the heteroaryl has 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl has 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl has 1 nitrogen atom. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the pyridyl is a 2-pyridyl. In some embodiments, the

pyridyl is a 3-pyridyl (  ). In some embodiments, the pyridyl is a 4-pyridyl. In

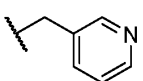
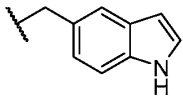
some embodiments, the indolyl is 5-indolyl (  ). In some embodiments, the heteroaryl is an imidazolyl, oxazolyl, thiazolyl, oxadiazolyl, thiadiazolyl, or triazolyl. In some embodiments, the haloalkyl is  $\text{CF}_3$ ,  $\text{CHF}_2$ ,  $\text{CH}_2\text{F}$ ,  $\text{CH}_2\text{CF}_3$ ,  $\text{CH}_2\text{CHF}_2$ , or  $\text{CF}_2\text{CF}_3$ .

**[0100]** In some embodiments, R<sup>2</sup> is not . In some embodiments, R<sup>2</sup> is not . In some embodiments, R<sup>2</sup> is not isopropyl.

**[0101]** In some embodiments, when R<sup>1</sup> is isopropyl, R<sup>2</sup> is not .

**[0102]** In some embodiments of Formula I, n is 0-2. In some embodiments, n is 1 or 2. In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, n is 2. In some embodiments, n is 3.

**[0103]** In some embodiments, of Formula I, R<sup>1</sup> is alkyl or aryl and R<sup>2</sup> is  $-(CH_2)_n$ -heteroaryl, wherein n is 1 or 2. In some embodiments, the alkyl is a C<sub>1-5</sub>alkyl. In some embodiments, the alkyl is ethyl or propyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is:

3-pyridyl (  ) or 5-indolyl (  ).

**[0104]** In some embodiments, of Formula I, X is -NH-, Y is -OH, R<sup>1</sup> is alkyl or aryl and R<sup>2</sup> is  $-(CH_2)_n$ -heteroaryl or  $-(CH_2)_n$ -heteroaryl, wherein n is 1 or 2. In some embodiments, the alkyl is a C<sub>1-5</sub>alkyl. In some embodiments, the alkyl is ethyl or propyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments, n is 2.

**[0105]** In some embodiments, of Formula I, X is -NH-, Y is -OH, R<sup>1</sup> is alkyl or aryl and R<sup>2</sup> is  $-(CH_2)_n$ -heteroaryl, wherein n is 1 or 2. In some embodiments, the alkyl is a C<sub>1-5</sub>alkyl. In some embodiments, the alkyl is ethyl or propyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the

heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments, n is 2.

**[0106]** In some embodiments of Formula I, R<sup>3</sup> is -OH, alkoxy, alkyl, -S-alkyl, -NH-alkyl, -N(CH<sub>3</sub>)-alkyl, -N(H)aralkyl, -O-haloalkyl, -O-aryl, -O-heteroaryl, or -O-aralkyl. In some embodiments, R<sup>3</sup> is -OH, alkoxy, -O-haloalkyl, -O-aryl, -O-heteroaryl, -O-aralkyl or -O-alkylene-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, R<sup>3</sup> is -OH, alkoxy, -O-haloalkyl, -O-aryl, -O-heteroaryl, or -O-aralkyl. In some embodiments, R<sup>3</sup> is OH, alkoxy, O-heteroaryl, or -NH-alkyl. In some embodiments, R<sup>3</sup> is -OH, alkoxy, or O-heteroaryl. In some embodiments, R<sup>3</sup> is -OH or alkoxy. In some embodiments, R<sup>3</sup> is -OH or -O-heteroaryl. In some embodiments, R<sup>3</sup> is -OH. In some embodiments, R<sup>3</sup> is -O-heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the pyridyl is a 3-pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the alkoxy is a C<sub>1-5</sub> alkoxy. In some embodiments, the alkoxy is a C<sub>2-5</sub> alkoxy. In some embodiments, the alkoxy is -OMe, -OEt, or -OiPr. In some embodiments, the alkoxy is -OMe. In some embodiments, the alkoxy is -OEt, -OPr, or -OiPr. In some embodiments, the alkoxy is -OEt. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, R<sup>3</sup> is -O-alkylene-NR<sup>5</sup>R<sup>6</sup> or -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, R<sup>3</sup> is -O-alkylene-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, the -O-alkylene-NR<sup>5</sup>R<sup>6</sup> is -O-CH<sub>2</sub>-C(O)-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, the alkylene is a C<sub>2-5</sub> alkylene, optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is an ethylene (-CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is a propylene (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

**[0107]** In some embodiments, of Formula I, R<sup>1</sup> is alkyl or aryl; R<sup>2</sup> is -(CH<sub>2</sub>)<sub>n</sub>-aryl or -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, wherein n is 1 or 2; and R<sup>3</sup> is -OH, -O-alkyl or -O-CH<sub>2</sub>-C(O)-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, each alkyl is independently a C<sub>1-5</sub>alkyl. In some embodiments,

each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments,  $R^3$  is  $-OH$ . In some embodiments,  $n$  is 2.

**[0108]** In some embodiments, of Formula I,  $R^1$  is alkyl or aryl;  $R^2$  is  $-(CH_2)_n$ -aryl or  $-(CH_2)_n$ -heteroaryl, wherein  $n$  is 1 or 2; and  $R^3$  is  $-OH$ . In some embodiments, each alkyl is independently a  $C_{1-5}$ alkyl. In some embodiments, each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments,  $R^3$  is  $-OH$ . In some embodiments,  $n$  is 2.

**[0109]** In some embodiments, of Formula I,  $R^1$  is alkyl or aryl;  $R^2$  is  $-(CH_2)_n$ -heteroaryl, wherein  $n$  is 1 or 2; and  $R^3$  is  $-OH$  or  $-O$ -alkyl. In some embodiments, each alkyl is independently a  $C_{1-5}$ alkyl. In some embodiments, each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments,  $R^3$  is  $OH$ . In some embodiments,  $n$  is 2.

**[0110]** In some embodiments, of Formula I,  $R^1$  is alkyl or aryl;  $R^2$  is  $-(CH_2)_n$ -heteroaryl, wherein  $n$  is 1 or 2; and  $R^3$  is  $-OH$ . In some embodiments, each alkyl is independently a  $C_{1-5}$ alkyl. In some embodiments, each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments,  $R^3$  is  $OH$ . In some embodiments,  $n$  is 2.

**[0111]** In some embodiments, of Formula I, X is -NH-, Y is -OH, R<sup>1</sup> is alkyl or aryl; R<sup>2</sup> is -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, wherein n is 1 or 2; and R<sup>3</sup> is -OH or -O-alkyl. In some embodiments, each alkyl is independently a C<sub>1-5</sub>alkyl. In some embodiments, each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments, R<sup>3</sup> is OH. In some embodiments, n is 2.

**[0112]** In some embodiments, of Formula I, X is -NH-, Y is -OH, R<sup>1</sup> is alkyl or aryl; R<sup>2</sup> is -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, wherein n is 1 or 2; and R<sup>3</sup> is -OH. In some embodiments, each alkyl is independently a C<sub>1-5</sub>alkyl. In some embodiments, each alkyl is independently methyl, ethyl or propyl. In some embodiments, the alkyl is ethyl. In some embodiments, the aryl is phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the aryl is 4-fluorophenyl. In some embodiments, the heteroaryl is pyridyl or indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl. In some embodiments, R<sup>3</sup> is OH. In some embodiments, n is 2.

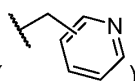
**[0113]** In some embodiments, of Formula I, R<sup>1</sup> is alkyl or aryl; R<sup>2</sup> is -CH<sub>2</sub>-alkyl; and R<sup>3</sup> is -OH, -O-alkyl or -O-CH<sub>2</sub>-C(O)-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, R<sup>1</sup> is C<sub>1-5</sub>-alkyl or phenyl. In some R<sup>1</sup> is ethyl or phenyl. In some embodiments, the phenyl is optionally substituted with one or more halogens. In some embodiments, the phenyl is 4-fluorophenyl. In some embodiments, the -CH<sub>2</sub>-alkyl is -CH<sub>2</sub>-aralkyl or -CH<sub>2</sub>-heteroaralkyl. In some embodiments, the heteroaralkyl is -CH<sub>2</sub>-pyridyl or -CH<sub>2</sub>-indolyl. In some embodiments, the heteroaralkyl is -CH<sub>2</sub>-(3-pyridyl) or -CH<sub>2</sub>-(5-indolyl). In some embodiments, R<sup>3</sup> is OH.

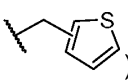
**[0114]** In some embodiments of Formula I, R<sup>3a</sup> is H. In some embodiments, R<sup>3a</sup> is halogen. In some embodiments, R<sup>3a</sup> is H or F.

**[0115]** In some embodiments of Formula I, R<sup>5</sup> is H, -C(O)alkyl, -C(O)cycloalkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl. In some embodiments, R<sup>5</sup> is H, -C(O)alkyl or -C(O)aralkyl. In some embodiments, R<sup>5</sup> is -C(O)aryl or -C(O)heteroaryl. In some embodiments, alkyl is a C<sub>1-5</sub> alkyl. In some embodiments, R<sup>5</sup> is alkyl, aralkyl, or heteroaralkyl. In some embodiments, the C<sub>1-5</sub> alkyl is Me, Et, propyl, butyl, or *i*Pr. In some embodiments, the C<sub>1-5</sub> alkyl is Me or Et. In some embodiments, the C<sub>1-5</sub> alkyl is

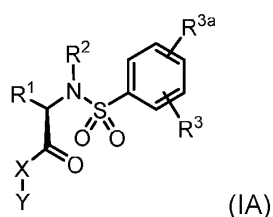
Me. In some embodiments, the cycloalkyl is a C<sub>3-6</sub> cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the aryl is a phenyl.

**[0116]** In some embodiments of Formula I, R<sup>6</sup> is H, alkyl, cycloalkyl, or aryl. In some embodiments, alkyl is a C<sub>1-5</sub> alkyl. In some embodiments, the C<sub>1-5</sub> alkyl is Me, Et, Pr, Bu, or *i*Pr. In some embodiments, the C<sub>1-5</sub> alkyl is Me or Et. In some embodiments, the C<sub>1-5</sub> alkyl is Me. In some embodiments, the alkyl is an aralkyl or heteroaralkyl. In some embodiments, the alkyl is an aralkyl. In some embodiments, the alkyl is a heteroaralkyl. In some embodiments, the aralkyl is –CH<sub>2</sub>aryl. In some embodiments, the aralkyl is –CH<sub>2</sub>phenyl. In some embodiments, the phenyl is optionally substituted with halogen, C<sub>1-5</sub>alkyl, –OC<sub>1-5</sub>alkyl, –SC<sub>1-5</sub>alkyl, fluoroalkyl (e.g., CF<sub>3</sub>, CF<sub>2</sub>H, CFH<sub>2</sub>, and the like), or phenyl. In some embodiments, the heteroaralkyl is

–CH<sub>2</sub>heteroaralkyl. In some embodiments, the heteroaralkyl is –CH<sub>2</sub>pyridyl (  )

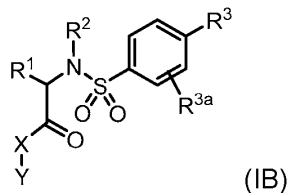
or –CH<sub>2</sub>thiophenyl (  ). In some embodiments, the heteroaralkyl is –CH<sub>2</sub>pyridyl. In some embodiments, the –CH<sub>2</sub>pyridyl is –CH<sub>2</sub>-(2-pyridyl) or –CH<sub>2</sub>-(3-pyridyl). In some embodiments, the –CH<sub>2</sub>thiophenyl is –CH<sub>2</sub>-(2-thiophenyl). In some embodiments, the cycloalkyl is a C<sub>3-6</sub> cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the aryl is a phenyl.

**[0117]** In some embodiments, the compound has the structure of Formula IA:



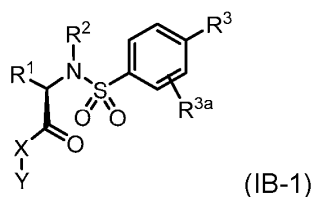
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>3a</sup> are as described above in Formula I.

**[0118]** In some embodiments, the compound has the structure of Formula IB:



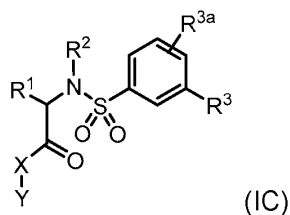
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>3a</sup> are as described above in Formula I.

**[0119]** In some embodiments, the compound has the structure of Formula IB-1:



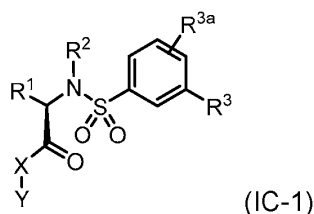
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>3a</sup> are as described above in Formula I.

**[0120]** In some embodiments, the compound has the structure of Formula IC:



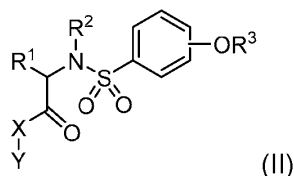
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, and R<sup>3a</sup> are as described above in Formula I.

**[0121]** In some embodiments, the compound has the structure of Formula IC-1:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>3a</sup> are as described above in Formula I.

**[0122]** In some embodiments, the compound has the structure of Formula II:

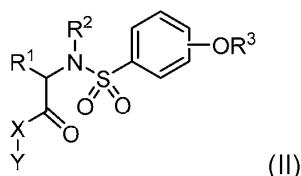


or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

R<sup>3</sup> is H, alkyl, -alkylene-NR<sup>5</sup>R<sup>6</sup>, haloalkyl, aryl, aralkyl, or heteroaryl; and

X, Y, R<sup>1</sup>, R<sup>2</sup>, R<sup>5</sup>, and R<sup>6</sup> are as described above in Formula I.

**[0123]** In some embodiments, the present disclosure provides a compound of Formula II:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is -NH-, -N(C<sub>1-5</sub> alkyl)-, or -N(CH<sub>2</sub>CF<sub>3</sub>)-, Y is -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>); and

when X is -O-, Y is H;

R<sup>1</sup> is alkyl, -alkylene-OH, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, heteroaralkyl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein R<sup>1</sup> is optionally substituted with one or more groups selected from -OH, -OMe, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>;

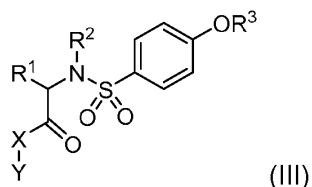
R<sup>2</sup> is H, -CH<sub>2</sub>-aryl, CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -CH<sub>2</sub>-heteroaryl, or -CH<sub>2</sub>-haloalkyl; and

R<sup>3</sup> is H, alkyl, -alkylene-NR<sup>5</sup>R<sup>6</sup>, haloalkyl, aryl, heteroaryl, or aralkyl, each of which is optionally substituted;

R<sup>5</sup> is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl; and

R<sup>6</sup> is H, alkyl, or aryl.

**[0124]** In some embodiments, the compound has the structure of Formula III:

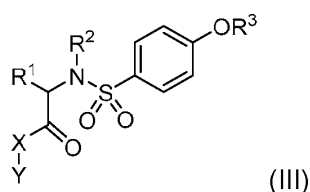


or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

R³ is H, alkyl, -alkylene-NR⁵R⁶, haloalkyl, aryl, aralkyl, or heteroaryl; and

X, Y, R¹, R², R⁵, and R⁶ are as described above in Formula I.

**[0125]** In some embodiments, the present disclosure provides a compound of Formula III:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C₁-₅ alkyl)-, -N(CH₂CF₃)-, or -O-;

Y is -OH, -OCH₃, -OCH₂CH₃, -NH₂, -NH(CH₃), -NH(CH₂CH₃), or -NH(CH₂CF₃);

R¹ is alkyl, -alkylene-OH, -alkylene-NH₂, -alkylene-C(=O)NH₂, heteroaralkyl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein R¹ is optionally substituted with one or more groups selected from -OH, halogen, -CHF₂, -CH₂F, or -CF₃;

R² is H, -CH₂-aryl, CH₂-alkyl, -CH(Me)-alkyl, -CH₂-heteroaryl, or -CH₂-haloalkyl;

and

R³ is H, alkyl, -alkylene-NR⁵R⁶, haloalkyl, aryl, heteroaryl, or aralkyl, each of which is optionally substituted;

R⁵ is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl; and

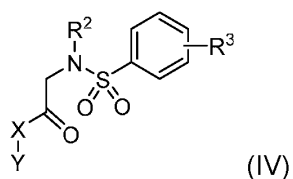
R⁶ is H, alkyl, or aryl.

**[0126]** In some embodiments of Formula II and Formula III, R² is -(CH₂)ₙ-aryl, -(CH₂)ₙ-heteroaryl, or -CH₂-alkyl. In some embodiments, R² is -CH₂-aryl, -CH₂-heteroaryl, or -CH₂-alkyl. In some embodiments, R² is -CH₂-alkyl. In some embodiments, the -CH₂-alkyl is optionally substituted with an aryl or heteroaryl. In some embodiments, the -CH₂-alkyl

is  $-\text{CH}_2\text{CH}_3$  substituted with aryl or heteroaryl. In some embodiments, the  $\text{CH}_2\text{CH}_3$  substituted with aryl is  $-\text{CH}_2\text{CH}_2\text{-aryl}$ . In some embodiments, the  $\text{CH}_2\text{CH}_3$  substituted with heteroaryl or  $-\text{CH}_2\text{CH}_2\text{-heteroaryl}$ . In some embodiments, the aryl is 3-fluorophenyl, 3-methoxyphenyl, 3-chlorophenyl, 4-fluorophenyl, 3-fluorophenyl, 3-difluoromethylphenyl, 3-thiomethylphenyl, 4-tolyl, or 3-tolyl. In some embodiments, the heteroaryl is pyridyl, indolyl, indazolyl, thiazolyl, or oxazolyl. In some embodiments, the pyridyl is 2-pyridyl or 3-pyridyl. In some embodiments, the pyridyl is 3-pyridyl. In some embodiments, the thiazolyl is 4-thiazolyl or 5-thiazolyl. In some embodiments, the oxazolyl is 4-oxazolyl or 5-oxazolyl. In some embodiments, the indolyl is 5-indolyl. In some embodiments, the heteroaryl is 3-pyridyl or 5-indolyl.

**[0127]** In some embodiments of Formula II and Formula III,  $\text{R}^3$  is H, alkyl, haloalkyl, or heteroaryl. In some embodiments,  $\text{R}^3$  is H, alkyl, or heteroaryl. In some embodiments,  $\text{R}^3$  is H or alkyl. In some embodiments,  $\text{R}^3$  is H. In some embodiments, the alkyl is a  $\text{C}_{1-5}$  alkyl. In some embodiments, the alkyl is a  $\text{C}_{2-5}$  alkyl. In some embodiments, the alkyl is Me, Et, or *i*Pr. In some embodiments, the alkyl is Me. In some embodiments, the alkyl is Et, Pr, or *i*Pr. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, the haloalkyl is  $\text{CF}_3$ ,  $\text{CHF}_2$ ,  $\text{CH}_2\text{F}$ ,  $\text{CH}_2\text{CF}_3$ , or  $\text{CF}_2\text{CF}_3$ . In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl. In some embodiments,  $\text{R}^3$  is H,  $\text{C}_{1-5}$  alkyl, or 5- or 6-membered heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the heteroaryl is an imidazolyl.

**[0128]** In some embodiments, the compound has the structure of Formula IV:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $-\text{NH}-$ ,  $-\text{N}(\text{C}_{1-5} \text{ alkyl})-$ ,  $-\text{N}(\text{CH}_2\text{CF}_3)-$ , or  $-\text{O}-$ ;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is -NH-, -N(C<sub>1-5</sub> alkyl)-, or -N(CH<sub>2</sub>CF<sub>3</sub>)-, Y is -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>); and

when X is -O-, Y is H;

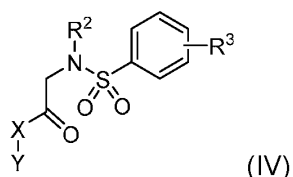
R<sup>2</sup> is -CH<sub>2</sub>-aryl, -CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -CH<sub>2</sub>-heteroaryl, or -CH<sub>2</sub>-haloalkyl;

R<sup>3</sup> is -OH, alkoxy, -O-alkylene-NR<sup>5</sup>R<sup>6</sup>, alkyl, -S-alkyl, -NH-alkyl, -N(CH<sub>3</sub>)-alkyl, -N(H)aralkyl, -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>, -O-haloalkyl, -O-aryl, -O-heteroaryl, or -O-aralkyl, each of which is optionally substituted;

R<sup>5</sup> is H, aralkyl, heteroaralkyl, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl; and

R<sup>6</sup> is H, alkyl, or aryl.

**[0129]** In some embodiments, the compound has the structure of Formula IV:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X, Y, and R<sup>3</sup> are as described above in Formula I; and

R<sup>2</sup> is alkyl.

**[0130]** In some embodiments of Formula IV, R<sup>2</sup> is a C<sub>1-6</sub> alkyl. In some embodiments, the alkyl is a C<sub>2-6</sub> alkyl. In some embodiments, the alkyl is methyl ethyl, propyl, isopropyl, butyl, isobutyl, isoamyl, or isopentyl. In some embodiments, the alkyl is methyl, ethyl, propyl, isopropyl, or isobutyl. In some embodiments, the alkyl is ethyl, propyl, or isobutyl. In some embodiments, alkyl is methyl or ethyl. In some embodiments, the C<sub>1-6</sub> alkyl is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>), -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or -CH<sub>2</sub>CHF<sub>2</sub>. In some embodiments, the C<sub>1-6</sub> alkyl is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>.

**[0131]** In some embodiments of Formula IV, R<sup>3</sup> is -OH, alkoxy, alkyl, -S-alkyl, -NH-alkyl, -N(CH<sub>3</sub>)-alkyl, -N(H)aralkyl, -O-haloalkyl, -O-aryl, -O-heteroaryl, or -O-aralkyl. In some embodiments, R<sup>3</sup> is OH, alkoxy, O-heteroaryl, or -NH-alkyl. In some embodiments, R<sup>3</sup> is

OH, alkoxy, or O-heteroaryl. In some embodiments,  $R^3$  is OH or alkoxy. In some embodiments,  $R^3$  is OH or O-heteroaryl. In some embodiments,  $R^3$  is OH. In some embodiments,  $R^3$  is O-heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the pyridyl is a 3-pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the alkoxy is a  $C_{1-5}$  alkoxy. In some embodiments, the alkoxy is a  $C_{2-5}$  alkoxy. In some embodiments, the alkoxy is OMe, OEt, or OiPr. In some embodiments, the alkoxy is OMe. In some embodiments, the alkoxy is OEt, OPr, or OiPr. In some embodiments, the alkoxy is OEt. In some embodiments, the alkyl is methyl or ethyl. In some embodiments,  $R^3$  is -O-alkylene- $NR^5R^6$  or -N(H)C(O)-alkylene- $NR^5R^6$ . In some embodiments,  $R^3$  is -O-alkylene- $NR^5R^6$ . In some embodiments, the -O-alkylene- $NR^5R^6$  is -O-CH<sub>2</sub>-C(O)- $NR^5R^6$ . In some embodiments, the alkylene is a  $C_{2-5}$  alkylene, optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is an ethylene (-CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is a propylene (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

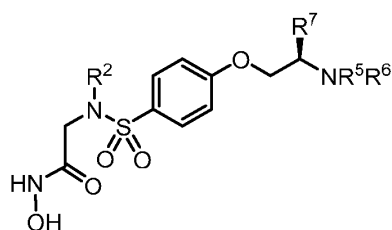
**[0132]** In some embodiments of Formula IV,  $R^3$  is -OH, alkoxy, -O-alkylene- $NR^5R^6$ , or -N(H)C(O)-alkylene- $NR^5R^6$ , wherein the alkylene is optionally substituted with an  $R^7$ . In some embodiments,  $R^3$  is -O-alkylene- $NR^5R^6$  or -N(H)C(O)-alkylene- $NR^5R^6$ , wherein the alkylene is optionally substituted with an  $R^7$ . In some embodiments, the alkylene is a  $C_{2-5}$  alkylene, optionally substituted with an  $R^7$ . In some embodiments, the alkylene is an ethylene (-CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with an  $R^7$ . In some embodiments, the alkylene is a propylene (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with an  $R^7$ . In some embodiments,  $R^7$  is oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments,  $R^7$  is F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

**[0133]** In some embodiments of Formula IV,  $R^5$  is H, aralkyl, heteroaralkyl, -C(O)alkyl, -C(O)cycloalkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl. In some embodiments,  $R^5$  is

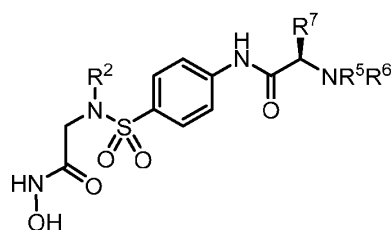
H, aralkyl, heteroaralkyl. In some,  $R^5$  is H, -C(O)alkyl, -C(O)cycloalkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl. In some embodiments,  $R^5$  is H, -C(O)alkyl or -C(O)aralkyl. In some embodiments,  $R^5$  is -C(O)aryl or -C(O)heteroaryl. In some embodiments, alkyl is a  $C_{1-5}$  alkyl. In some embodiments, the  $C_{1-5}$  alkyl is Me, Et, Pr, Bu, or *i*Pr. In some embodiments, the  $C_{1-5}$  alkyl is Me or Et. In some embodiments, the  $C_{1-5}$  alkyl is Me. In some embodiments, the cycloalkyl is a  $C_{3-6}$  cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the aryl is a phenyl. In some embodiments, the aralkyl is -CH<sub>2</sub>aryl. In some embodiments, the aralkyl is -CH<sub>2</sub>phenyl. In some embodiments, the aryl (e.g., phenyl) is optionally substituted with halogen,  $C_{1-5}$ alkyl, -OC<sub>1-5</sub>alkyl, -SC<sub>1-5</sub>alkyl, fluoroalkyl (e.g., CF<sub>3</sub>, CF<sub>2</sub>H, CFH<sub>2</sub>, and the like), or phenyl. In some embodiments, the heteroaralkyl is -CH<sub>2</sub>heteroaralkyl. In some embodiments, the heteroaralkyl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl. In some embodiments, the heteroaralkyl is -CH<sub>2</sub>pyridyl. In some embodiments, the -CH<sub>2</sub>pyridyl is -CH<sub>2</sub>-(2-pyridyl) or -CH<sub>2</sub>-(3-pyridyl). In some embodiments, the -CH<sub>2</sub>thiophenyl is -CH<sub>2</sub>-(2-thiophenyl).

**[0134]** In some embodiments of Formula IV,  $R^6$  is H, alkyl, cycloalkyl, or aryl. In some embodiments, alkyl is a  $C_{1-5}$  alkyl. In some embodiments, the  $C_{1-5}$  alkyl is Me, Et, propyl, butyl, or *i*Pr. In some embodiments, the  $C_{1-5}$  alkyl is Me or Et. In some embodiments, the  $C_{1-5}$  alkyl is Me. In some embodiments, the cycloalkyl is a  $C_{3-6}$  cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the aryl is a phenyl.

**[0135]** In some embodiments, the compound of Formula IV has a structure according to:



(IVA)



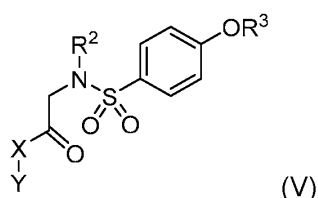
(IVB),

or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

$R^2$ ,  $R^5$ , and  $R^6$  are as defined above in Formulas I and IV; and

$R^7$  is F, oxo, alkyl, fluoroalkyl, aryl,  $-\text{CH}_2\text{-aryl}$ , or  $-\text{CH}_2\text{-heteroaryl}$ .

**[0136]** In some embodiments, the compound has the structure of Formula V:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

$R^3$  is H, alkyl,  $-\text{alkylene-NR}^5\text{R}^6$ , haloalkyl, aryl, aralkyl, or heteroaryl; and

X, Y,  $R^2$ ,  $R^5$ , and  $R^6$  are as described above in Formula I.

**[0137]** In some embodiments of Formula V,  $R^2$  is a  $\text{C}_{1-6}$  alkyl. In some embodiments, the alkyl is a  $\text{C}_{2-6}$  alkyl. In some embodiments, the alkyl is methyl ethyl, propyl, isopropyl, butyl, isobutyl, isoamyl, or isopentyl. In some embodiments, the alkyl is methyl, ethyl, propyl, isopropyl, or isobutyl. In some embodiments, the alkyl is ethyl, propyl, or isobutyl. In some embodiments, alkyl is methyl or ethyl.

**[0138]** In some embodiments of Formula V,  $R^2$  is  $-\text{CH}_2\text{-alkyl}$ ,  $-\text{CH}_2\text{-aryl}$ , or  $-\text{CH}_2\text{-heteroaryl}$ . In some embodiments,  $R^2$  is  $-\text{CH}_2\text{-alkyl}$ . In some embodiments, the  $-\text{CH}_2\text{-alkyl}$  is  $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ ,  $-\text{CH}_2\text{CH}_2\text{CH}_3$ ,  $-\text{CH}_2\text{CH}(\text{CH}_3)(\text{CH}_2\text{CH}_3)$ , or  $-\text{CH}_2\text{CHF}_2$ . In some embodiments, the  $-\text{CH}_2\text{-alkyl}$  is  $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ . In some embodiments, the  $-\text{CH}_2\text{-aryl}$  is  $-\text{CH}_2\text{-Ph}$ . In some embodiments, the  $-\text{CH}_2\text{-heteroaryl}$  is  $-\text{CH}_2\text{-pyridyl}$ . In some embodiments, the pyridyl is 3-pyridyl.

**[0139]** In some embodiments of Formula V,  $R^3$  is  $-\text{OH}$ , alkoxy, alkyl,  $-\text{S-alkyl}$ ,  $-\text{NH-alkyl}$ ,  $-\text{N}(\text{CH}_3)\text{-alkyl}$ ,  $-\text{N}(\text{H})\text{aralkyl}$ ,  $-\text{O-haloalkyl}$ ,  $-\text{O-aryl}$ ,  $-\text{O-heteroaryl}$ , or  $-\text{O-aralkyl}$ . In some embodiments,  $R^3$  is  $\text{OH}$ , alkoxy,  $\text{O-heteroaryl}$ , or  $-\text{NH-alkyl}$ . In some embodiments,  $R^3$  is  $\text{OH}$ , alkoxy, or  $\text{O-heteroaryl}$ . In some embodiments,  $R^3$  is  $\text{OH}$  or alkoxy. In some embodiments,  $R^3$  is  $\text{OH}$  or  $\text{O-heteroaryl}$ . In some embodiments,  $R^3$  is  $\text{OH}$ . In some embodiments,  $R^3$  is  $\text{O-heteroaryl}$ . In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and

S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the pyridyl is a 3-pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the alkoxy is a C<sub>1-5</sub> alkoxy. In some embodiments, the alkoxy is a C<sub>2-5</sub> alkoxy. In some embodiments, the alkoxy is OMe, OEt, or OiPr. In some embodiments, the alkoxy is OMe. In some embodiments, the alkoxy is OEt, OPr, or OiPr. In some embodiments, the alkoxy is OEt. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, R<sup>3</sup> is -O-alkylene-NR<sup>5</sup>R<sup>6</sup> or -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>. In some embodiments, the alkylene is a C<sub>2-5</sub> alkylene, optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is an ethylene (-CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, the alkylene is a propylene (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-), optionally substituted with oxo, F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

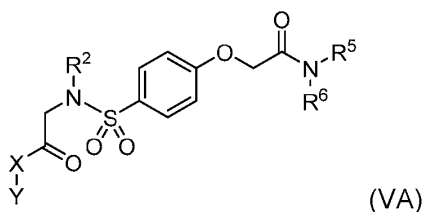
**[0140]** In some embodiments of Formula V, R<sup>3</sup> is H, alkyl, haloalkyl, or heteroaryl. In some embodiments, R<sup>3</sup> is H, alkyl, or heteroaryl. In some embodiments, R<sup>3</sup> is H or heteroaryl. In some embodiments, R<sup>3</sup> is H. In some embodiments R<sup>3</sup> is alkyl. In some embodiments, R<sup>3</sup> is heteroaryl. In some embodiments, the alkyl is a C<sub>1-5</sub> alkyl. In some embodiments, the alkyl is a C<sub>2-5</sub> alkyl. In some embodiments, the alkyl is Me, Et, or iPr. In some embodiments, the alkyl is Me. In some embodiments, the alkyl Et, Pr, or iPr. In some embodiments, the alkyl is methyl or ethyl. In some embodiments, the haloalkyl is CF<sub>3</sub>, CHF<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>CF<sub>3</sub>, or CF<sub>2</sub>CF<sub>3</sub>. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the pyridyl is a 3-pyridyl. In some embodiments, the heteroaryl is an imidazolyl.

**[0141]** In some embodiments of Formula V, R<sup>5</sup> is H, aralkyl, heteroaralkyl, -C(O)alkyl, -C(O)cycloalkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl. In some embodiments, R<sup>5</sup> is H, aralkyl, heteroaralkyl. In some, R<sup>5</sup> is H, -C(O)alkyl, -C(O)cycloalkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl. In some embodiments, R<sup>5</sup> is H, -C(O)alkyl or -C(O)aralkyl. In some embodiments, R<sup>5</sup> is -C(O)aryl or -C(O)heteroaryl. In some

embodiments, alkyl is a C<sub>1-5</sub> alkyl. In some embodiments, the C<sub>1-5</sub> alkyl is Me, Et, Pr, Bu, or *i*Pr. In some embodiments, the C<sub>1-5</sub> alkyl is Me or Et. In some embodiments, the C<sub>1-5</sub> alkyl is Me. In some embodiments, the cycloalkyl is a C<sub>3-6</sub> cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1 or 2 nitrogen atoms. In some embodiments, the heteroaryl is a pyridyl. In some embodiments, the heteroaryl is an imidazolyl. In some embodiments, the aryl is a phenyl.

**[0142]** In some embodiments of Formula V, R<sup>6</sup> is H, alkyl, cycloalkyl, or aryl. In some embodiments, alkyl is a C<sub>1-5</sub> alkyl. In some embodiments, the C<sub>1-5</sub> alkyl is Me, Et, propyl, butyl, or *i*Pr. In some embodiments, the C<sub>1-5</sub> alkyl is Me or Et. In some embodiments, the C<sub>1-5</sub> alkyl is Me. In some embodiments, the cycloalkyl is a C<sub>3-6</sub> cycloalkyl. In some embodiments, the cycloalkyl is a cyclopropyl. In some embodiments, the aryl is a phenyl.

**[0143]** In some embodiments, the compound has the structure of Formula VA:

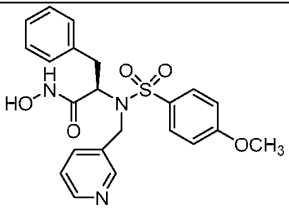
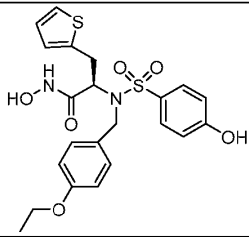
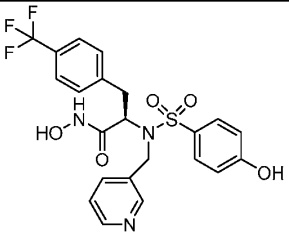
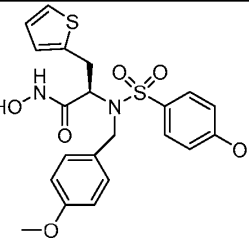
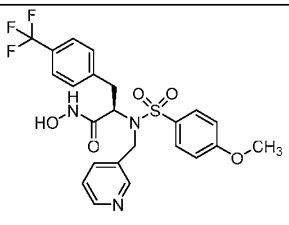
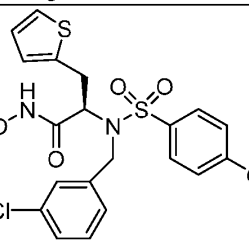
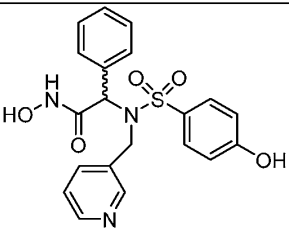
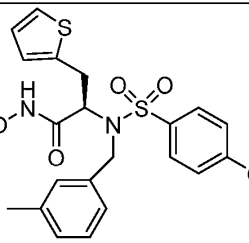
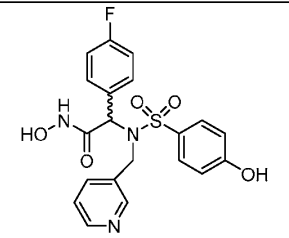
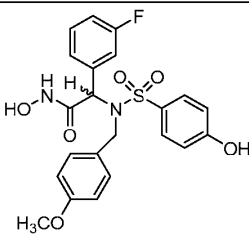
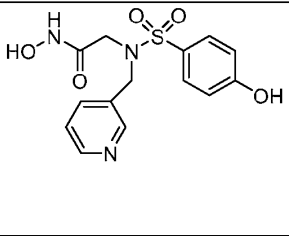
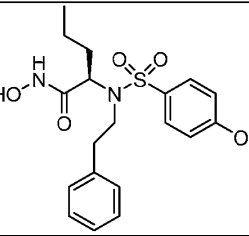


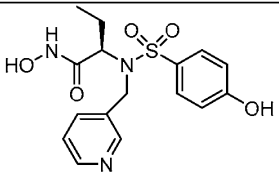
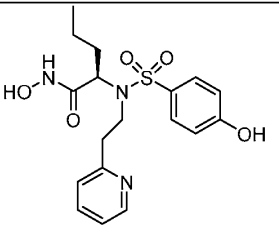
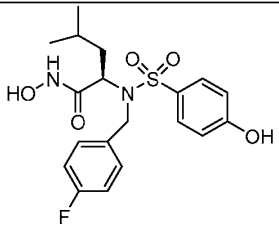
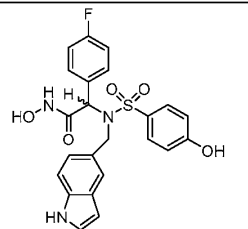
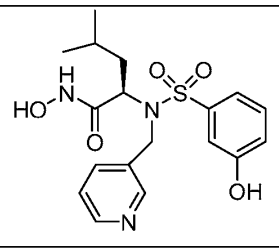
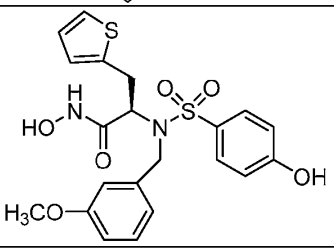
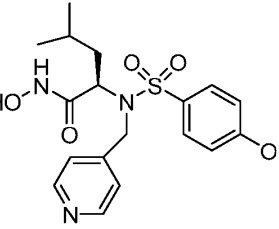
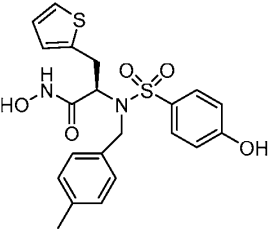
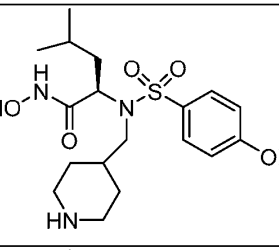
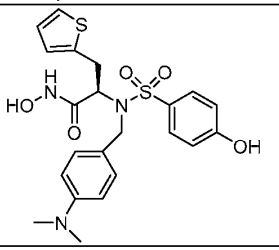
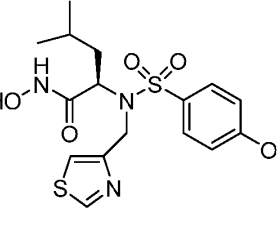
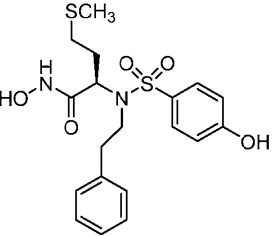
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein: X, Y, R<sup>2</sup>, R<sup>5</sup>, and R<sup>6</sup> are as described above in Formula I.

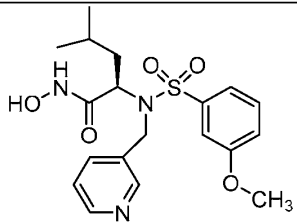
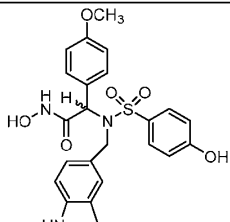
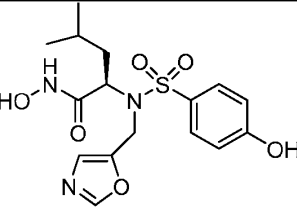
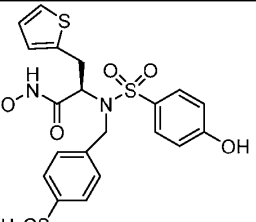
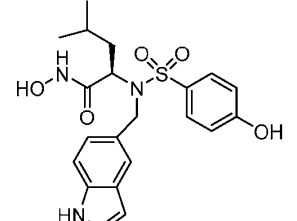
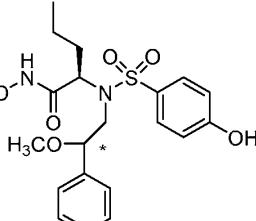
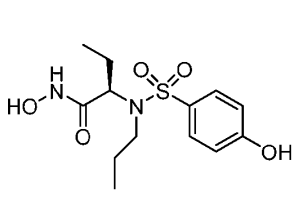
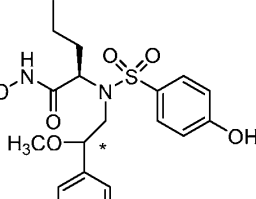
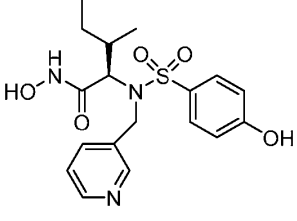
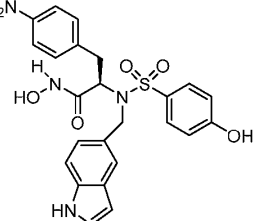
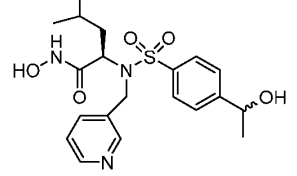
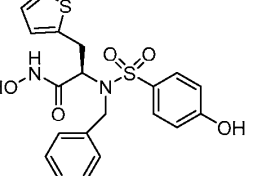
**[0144]** In some embodiments of Formula VA, R<sup>2</sup> is -CH<sub>2</sub>-alkyl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl. In some embodiments, R<sup>2</sup> is -CH<sub>2</sub>-alkyl. In some embodiments, the -CH<sub>2</sub>-alkyl is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>), or -CH<sub>2</sub>CHF<sub>2</sub>. In some embodiments, the -CH<sub>2</sub>-alkyl is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>. In some embodiments, -CH<sub>2</sub>-aryl is -CH<sub>2</sub>Ph. In some embodiments, the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from N, S, and O. In some embodiments, -CH<sub>2</sub>-heteroaryl is -CH<sub>2</sub>-pyridyl.

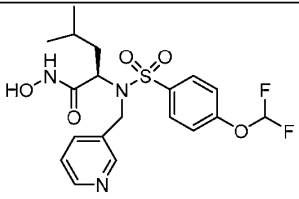
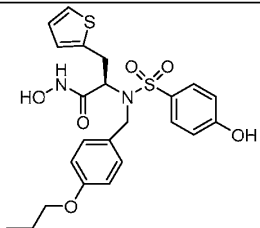
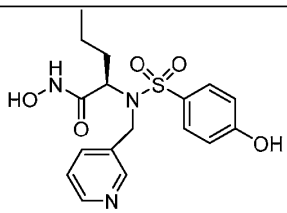
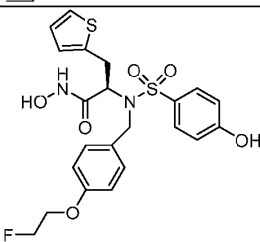
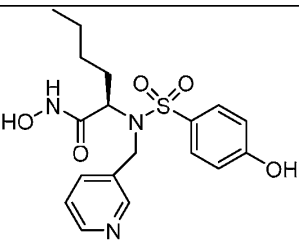
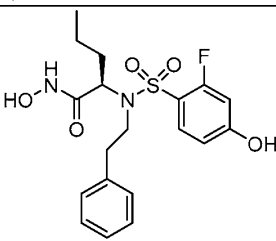
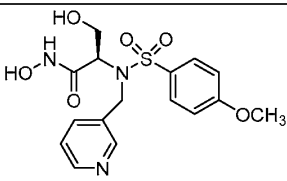
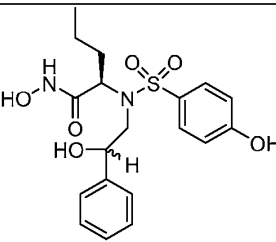
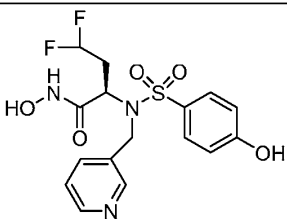
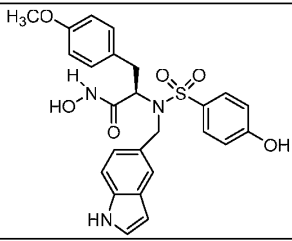
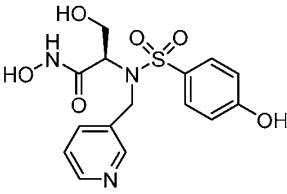
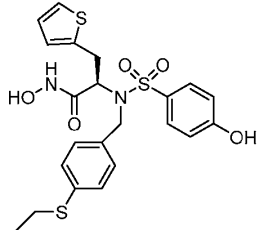
**[0145]** In some embodiments, the compound of the present disclosure (e.g., a compound of Formula (I)) is selected from the group consisting of:

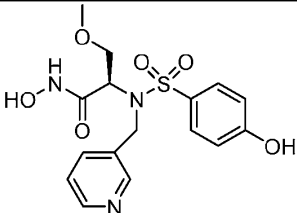
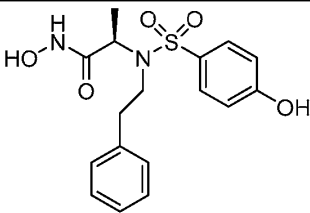
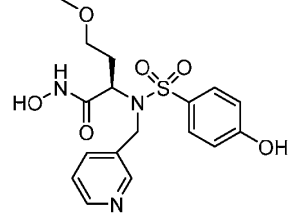
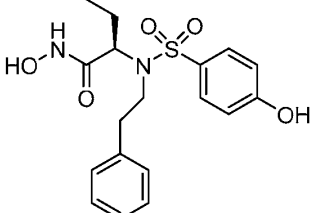
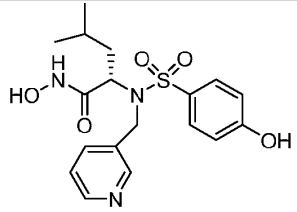
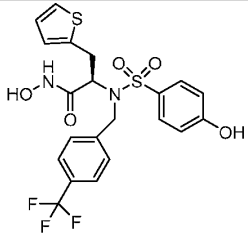
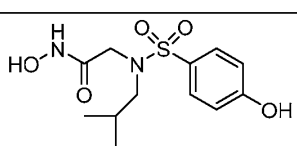
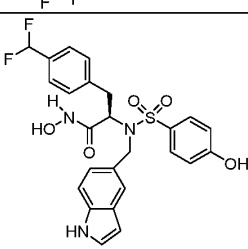
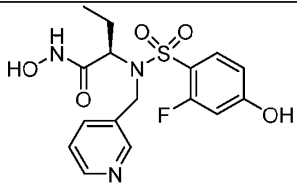
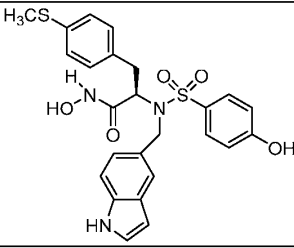
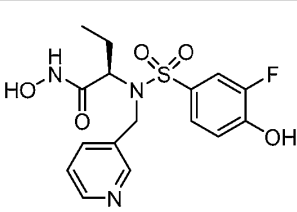
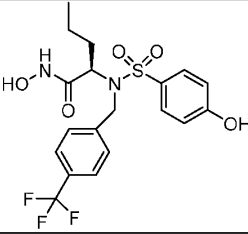
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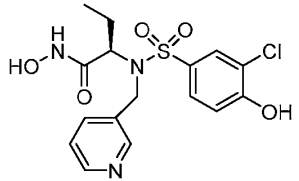
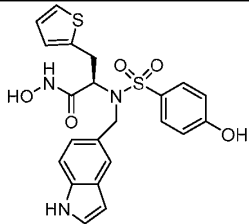
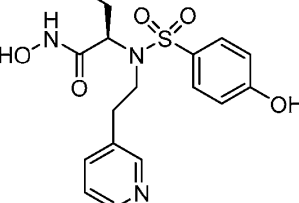
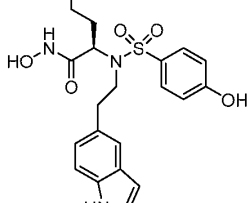
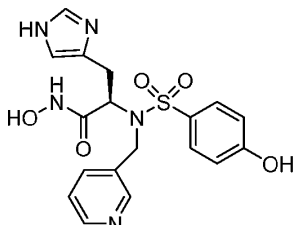
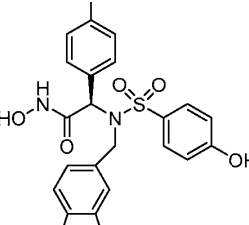
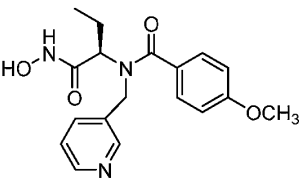
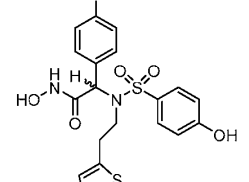
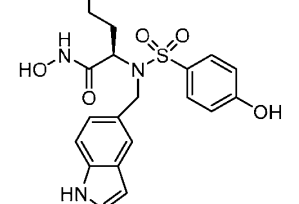
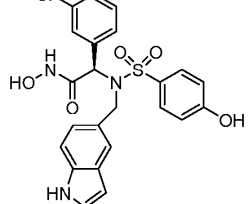
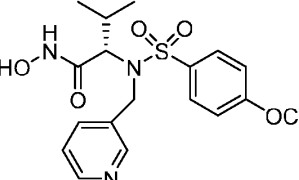
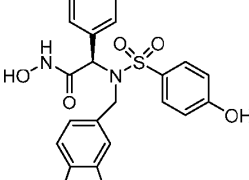
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|----|-------------------------------------------------------------------------------------|----|--------------------------------------------------------------------------------------|
| 19 |    | 81 |    |
| 20 |    | 82 |    |
| 21 |   | 83 |   |
| 22 |  | 84 |  |
| 23 |  | 85 |  |
| 24 |  | 86 |  |

|    |                                                                                     |    |                                                                                      |
|----|-------------------------------------------------------------------------------------|----|--------------------------------------------------------------------------------------|
| 25 |    | 87 |    |
| 26 |    | 88 |    |
| 27 |   | 89 |   |
| 28 |  | 90 |  |
| 29 |  | 91 |  |
| 30 |  | 92 |  |

|    |                                                                                     |    |                                                                                      |
|----|-------------------------------------------------------------------------------------|----|--------------------------------------------------------------------------------------|
| 31 |    | 93 |    |
| 32 |    | 94 |    |
| 33 |   | 95 |   |
| 35 |  | 96 |  |
| 36 |  | 97 |  |
| 37 |  | 98 |  |

|    |                                                                                     |     |                                                                                      |
|----|-------------------------------------------------------------------------------------|-----|--------------------------------------------------------------------------------------|
| 40 |    | 99  |    |
| 42 |    | 100 |    |
| 44 |   | 101 |   |
| 45 |  | 102 |  |
| 48 |  | 103 |  |
| 50 |  | 104 |  |

|    |                                                                                     |     |                                                                                      |
|----|-------------------------------------------------------------------------------------|-----|--------------------------------------------------------------------------------------|
| 53 |    | 105 |    |
| 54 |    | 106 |    |
| 55 |   | 107 |   |
| 56 |  | 108 |  |
| 57 |  | 109 |  |
| 58 |  | 110 |  |

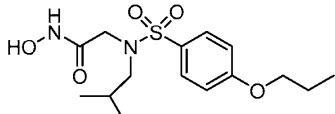
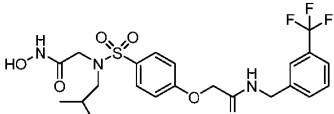
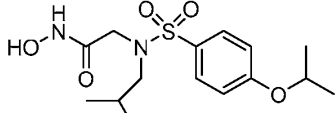
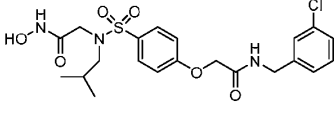
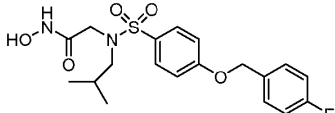
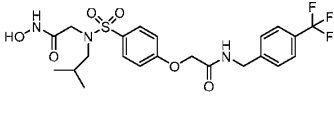
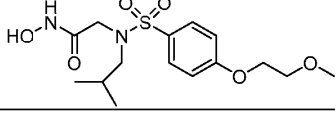
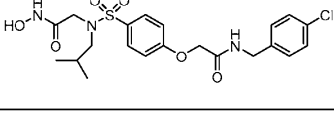
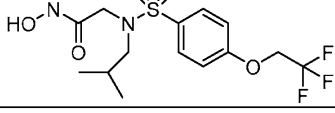
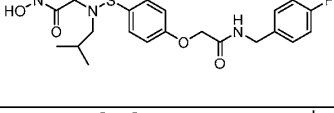
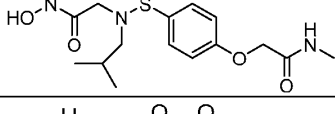
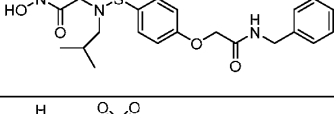
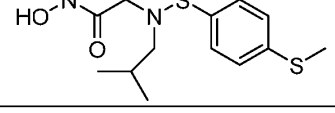
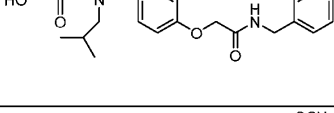
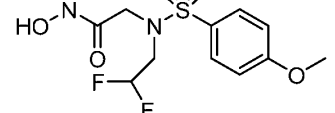
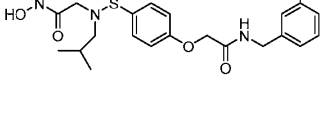
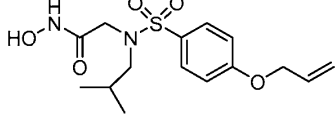
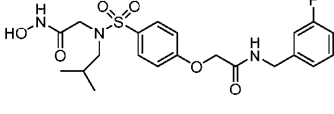
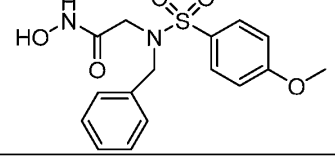
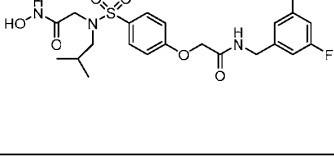
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|----|-------------------------------------------------------------------------------------|-----|--------------------------------------------------------------------------------------|
| 59 |    | 111 |    |
| 60 |    | 112 |    |
| 61 |   | 113 |   |
| 62 |  | 114 |  |
| 63 |  | 115 |  |
| 64 |  | 116 |  |

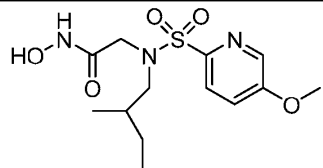
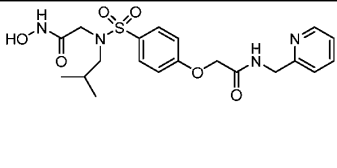
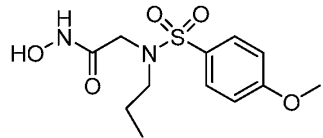
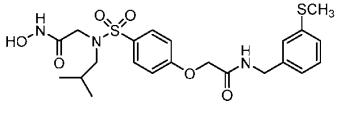
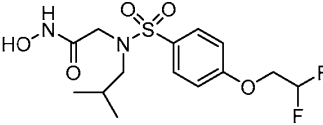
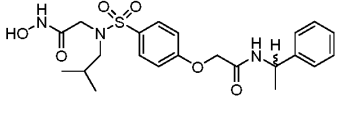
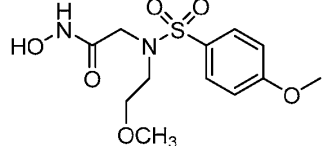
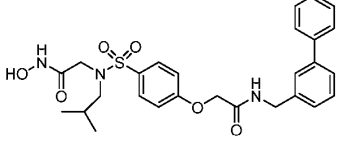
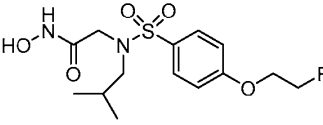
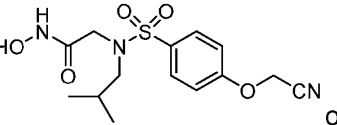
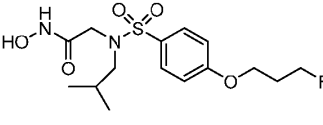
|    |  |     |  |
|----|--|-----|--|
| 65 |  | 117 |  |
| 66 |  | 118 |  |
| 67 |  | 119 |  |

or pharmaceutically acceptable salt thereof.

**[0146]** In some embodiments, the compound of the present disclosure (e.g., a compound of Formula (IV) or Formula (V)) is:

| Cmpd. #* | Structure | Cmpd. #* | Structure |
|----------|-----------|----------|-----------|
| A        |           | AC       |           |
| B        |           | AD       |           |
| C        |           | AE       |           |

|          |                                                                                     |           |                                                                                      |
|----------|-------------------------------------------------------------------------------------|-----------|--------------------------------------------------------------------------------------|
| <b>D</b> |    | <b>AF</b> |    |
| <b>E</b> |    | <b>AG</b> |    |
| <b>F</b> |    | <b>AH</b> |    |
| <b>G</b> |    | <b>AI</b> |    |
| <b>J</b> |    | <b>AJ</b> |    |
| <b>K</b> |  | <b>AK</b> |  |
| <b>M</b> |  | <b>AL</b> |  |
| <b>P</b> |  | <b>AM</b> |  |
| <b>R</b> |  | <b>AN</b> |  |
| <b>S</b> |  | <b>AO</b> |  |

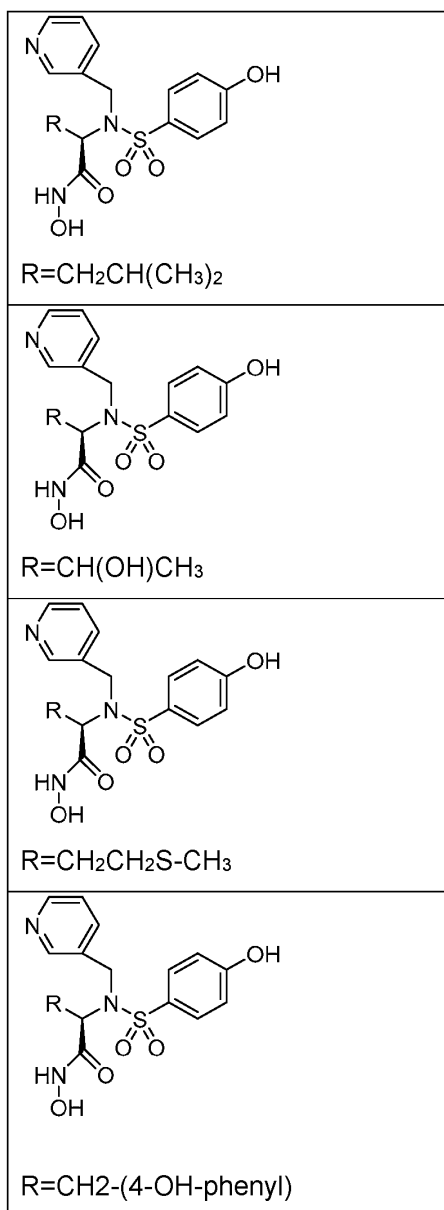
|           |                                                                                     |           |                                                                                     |
|-----------|-------------------------------------------------------------------------------------|-----------|-------------------------------------------------------------------------------------|
| <b>T</b>  |    | <b>AP</b> |   |
| <b>U</b>  |    | <b>AQ</b> |   |
| <b>X</b>  |    | <b>AR</b> |   |
| <b>Y</b>  |    | <b>AS</b> |   |
| <b>Z</b>  |   | <b>AT</b> |  |
| <b>AB</b> |  |           |                                                                                     |

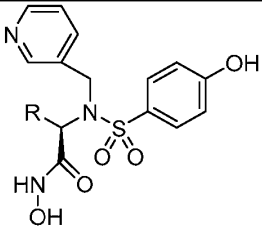
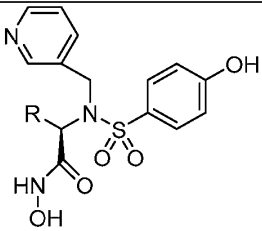
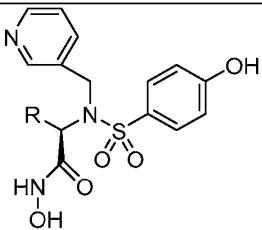
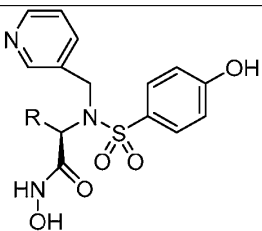
or pharmaceutically acceptable salt thereof.

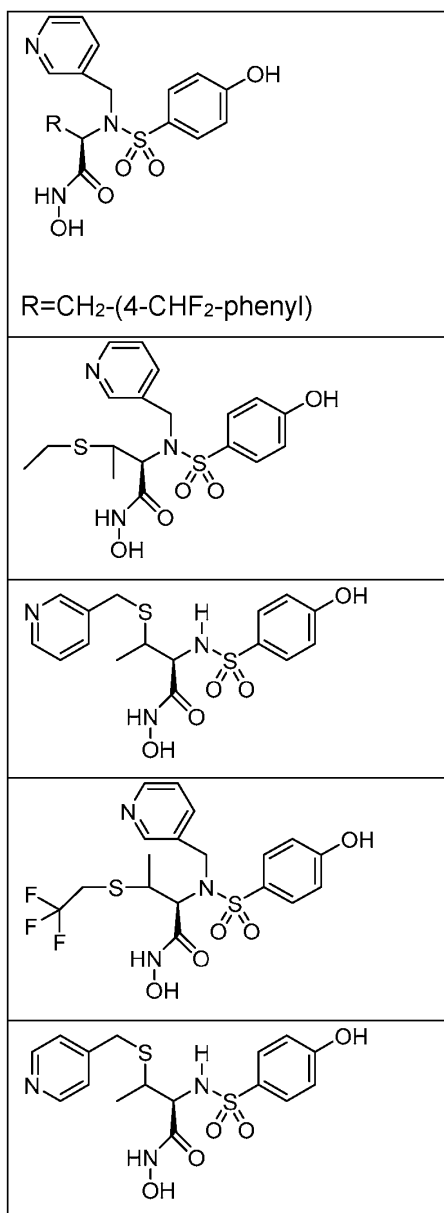
**[0147]** In some embodiments, the compound of the present disclosure (e.g., a compound of Formula I) is a compound in Table 7. In some embodiments, the compound of the present disclosure is a stereoisomer or a pharmaceutically acceptable salt of any of the compounds listed in Table 7.

**Table 7.** Exemplary small molecule inhibitors

Structure



|                                                                                     |
|-------------------------------------------------------------------------------------|
|    |
| R=CH <sub>2</sub> -phenyl                                                           |
|    |
| R=CH <sub>2</sub> -(3-indolyl)                                                      |
|   |
| R=CH <sub>2</sub> -(4-imidazolyl)                                                   |
|  |
| R=CH <sub>2</sub> -C(=O)NH <sub>2</sub>                                             |



**[0148]** In some embodiments, the compound of the present disclosure is a compound in Table 10. In some embodiments, the compound of the present disclosure is a stereoisomer or a pharmaceutically acceptable salt of a compound listed in Table 10.

**[0149]** In some embodiments, the compound of the present disclosure is a compound in Table 11. In some embodiments, the compound of the present disclosure is a stereoisomer or a pharmaceutically acceptable salt of a compound listed in Table 11.

**[0150]** Pharmaceutically acceptable derivatives of a compound may include salts, esters, enol ethers, enol esters, acetals, ketals, orthoesters, hemiacetals, hemiketals, acids, bases, solvates, hydrates or prodrugs thereof. Pharmaceutically acceptable salts include, but are not limited to, amine salts, such as but not limited to N,N'-dibenzylethylenediamine, chlorprocaine, choline, ammonia, diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium; transition metal salts, such as but not limited to zinc; and inorganic salts, such as but not limited to, sodium hydrogen phosphate and disodium phosphate; and also including, but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates, mesylates, and fumarates. Pharmaceutically acceptable esters include, but are not limited to, alkyl, alkenyl, alkynyl, aryl, aralkyl, and cycloalkyl esters of acidic groups, including, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids. Pharmaceutically acceptable enol ethers include, but are not limited to, derivatives of formula  $C=C(OR)$  where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, aralkyl and cycloalkyl. Pharmaceutically acceptable enol esters include, but are not limited to, derivatives of formula  $C=C(OC(O)R)$  where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, aralkyl and cycloalkyl. Pharmaceutically acceptable solvates and hydrates are complexes of a compound with one or more solvent or water molecules, or 1 to about 100, or 1 to about 10, or one to about 2, 3 or 4, solvent or water molecules.

**[0151]** A compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) may contain a chiral center. Such

chiral center may be either of the (R) or (S) configuration, or may be a mixture thereof. The compound may be enantiomerically pure, or may be stereoisomeric or diastereomeric mixtures. In embodiments wherein the compound undergoes epimerization *in vivo*, administration of a compound in its (R) form is equivalent to administration of the compound in its (S) form.

**[0152]** In some embodiments, the compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) has an inhibition constant ( $K_i$ ) of less than about 100 mM, less than about 10 mM, less than about 1 mM, less than about 0.1 mM, less than about 0.01 mM, less than about 0.001 mM, less than about 0.0001 mM, or less than about 0.00001 mM. In some embodiments, the compound has an inhibition constant in the range of about  $10^{-5}$  to about  $10^{-13}$  M, such as about  $10^{-5}$ , about  $10^{-6}$ , about  $10^{-7}$ , about  $10^{-8}$ , about  $10^{-9}$ , about  $10^{-10}$ , about  $10^{-11}$ , about  $10^{-12}$ , about  $10^{-13}$  M. The term "inhibition constant" denotes the concentration of inhibitor required to produce half maximum inhibition of an enzyme.

**[0153]** In some embodiments, the compound of the present disclosure has an  $IC_{50}$  of less than about 100 mM, less than about 10 mM, less than about 1 mM, less than about 0.1 mM, less than about 0.01 mM, less than about 0.001 mM, less than about 0.0001 mM, or less than about 0.00001 mM. In some embodiments, a compound has an  $IC_{50}$  in the range of about 1  $\mu$ M to about 500  $\mu$ M. In some embodiments, a compound has an  $IC_{50}$  in the range of about 0.1 to about 10 nM, about 10 nM to about 100 nM, about 100 nM to about 500 nM, about 500 nM to about 1  $\mu$ M, about 1  $\mu$ M to about 10  $\mu$ M, about 10  $\mu$ M to about 100  $\mu$ M, about 100  $\mu$ M to about 500  $\mu$ M, about 500  $\mu$ M to about 1 mM, or about 1 mM to about 100 mM. As used herein,  $IC_{50}$  is the half maximal inhibitor concentration (i.e., a measure of the potency of a substance in inhibiting a specific biological or biochemical function.)  $IC_{50}$  may be determined using standard inhibition assays known in the art. For example in some embodiments, the  $IC_{50}$  of a small molecule inhibitor may be determined by measuring cleavage of a FRET-based peptide substrate. The FRET-based peptide substrate may be, for example, Anaspec AS-27077, which has the sequence Mca - Pro - Leu - Gly - Leu - Dap(Dnp) - Ala - Arg -  $NH_2$  (SEQ ID NO: 10),

wherein Mca stands for 7-methoxy-coumarin-4-yl acetic acid-2,4-dinitrophenyl-lysine, and Dap(Dnp) stands for N<sup>β</sup>-2,4-dinitrophenyl-L-di-aminopropionic acid.

### **Pharmaceutical Compositions**

**[0154]** Also provided herein are pharmaceutical compositions comprising one or more compounds of the present disclosure. In some embodiments, a pharmaceutical composition comprises one or more compounds disclosed herein (e.g., a compound of Formula I, Formula IA, Formula IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) and one or more pharmaceutically acceptable carriers or excipients. A non-limiting list of pharmaceutically acceptable carriers and excipients is disclosed in Adejare, A. (Ed.). (2020) *Remington: The Science and Practice of Pharmacy*, 23<sup>rd</sup> Edition. Elsevier, which is hereby incorporated by reference in its entirety for all purposes.

**[0155]** A pharmaceutical composition can be prepared using conventional pharmaceutically acceptable excipients and additives and conventional techniques. Such pharmaceutically acceptable excipients and additives include, but are not limited to, non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, anti-oxidants, lubricants, flavorings, thickeners, coloring agents, emulsifiers and the like.

**[0156]** In some embodiments, the concentration of the inhibitor in the pharmaceutical composition range from about 1 nanomolar to about 1 micromolar, from about 1 micromolar to about 1 millimolar, or from about 1 millimolar to about 1 molar. In some embodiments, the concentration of the inhibitor is about 10 micromolar, about 25 micromolar, about 50 micromolar, about 75 micromolar, about 100 micromolar, about 250 micromolar, or about 500 micromolar.

**[0157]** The pharmaceutical composition can be formulated for administration systemically or locally. In some embodiments, the pharmaceutical composition is formulated for administration orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenously, intraarterially, intragastrically, nasally, intraperitoneally, subcutaneously, intramuscularly, intranasally, intrathecally, and intraarticularly or combinations thereof. In some embodiments, the pharmaceutical composition can be formulated for oral administration.

In some embodiments, the pharmaceutical composition can be formulated for intravenous administration.

**[0158]** For oral administration, the pharmaceutical compositions can take the form of, for example, tablets, capsules, or lozenges, prepared by conventional means with pharmaceutically acceptable excipients. In some embodiments, the pharmaceutical composition is formulated as a liquid. Liquid preparations can take the form of, for example, elixirs, solutions, syrups or suspensions, or they can be presented as dry product for constitution with water or other suitable vehicle before use. Oral administration also includes enteric formulations, which may include acid stable agents that maintain activity under gastrointestinal conditions, enteric coatings of pills, and the like, where there is a significant activity of the agent in intestinal tissues.

**[0159]** Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. The injectables, solutions and emulsions can also contain one or more excipients. Excipients include, for example, water, saline, dextrose, glycerol or ethanol. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, stabilizers, solubility enhancers, and other such agents, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate and cyclodextrins.

**[0160]** In some embodiments, the pharmaceutical composition is formulated for intranasal administration. Numerous delivery devices are available for intranasal administration such as instillation catheters, droppers, unit-dose containers, squeeze bottles pump sprays, airless and preservative-free sprays, compressed air nebulizers, metered-dose inhalers, insufflators and pressurized metered dose inhalers. Devices vary in accuracy of delivery, dose reproducibility, cost, and ease of use. Currently, metered-dose systems provide the greatest dose accuracy and reproducibility.

### **Methods of Treatment**

**[0161]** The present disclosure relates to methods of treating or preventing a disease or disorder in a subject, the method comprising administering a therapeutically effective

amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1 Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) or composition thereof to the subject in need thereof.

**[0162]** In some embodiments, the present disclosure provides a method of treating an inflammatory bowel disease or disorder in a subject, the method comprising administering a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) or composition thereof to the subject in need thereof. In some embodiments, the inflammatory bowel disease or disorder is Crohn's disease or ulcerative colitis. In some embodiments, the methods of the disclosure may be used to treat ulcerative colitis, indeterminate colitis, microscopic colitis and collagenous colitis.

**[0163]** In some embodiments, the present disclosure provides a method of treating cancer in a subject, the method comprising administering a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) or composition thereof to the subject in need thereof. In some embodiments, the cancer is a gastrointestinal (GI) cancer. The GI cancer may be, for example, esophageal cancer, gallbladder cancer, liver cancer, pancreatic cancer, stomach cancer, cancer of the small intestine, colorectal cancer, and anal cancer. In some embodiments, the cancer is colorectal cancer, such as adenocarcinoma, gastrointestinal stromal tumors (GIST), colorectal lymphoma, carcinoids, Turcot Syndrome, Peutz-Jeghers Syndrome (PJS), Familial Colorectal Cancer (FCC), or Juvenile Polyposis Coli. The cancer may be stage I, stage II, stage III, or stage IV (i.e., metastatic).

**[0164]** In some embodiments, the present disclosure provides a method of treating a systemic bacterial infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula

IVB, Formula V, or Formula VA) or composition thereof to the subject in need thereof. In some embodiments, the systemic bacterial infection is a systemic tissue infection. In some embodiments, the systemic bacterial infection is endocarditis or a urinary tract infection. In some embodiments, the systemic bacterial infection is septicemia.

**[0165]** In some embodiments of the disclosed methods, the subject is colonized by one or more pathogenic bacterial strain. Colonization may result in an acute infection, or result in a chronic infection. In some embodiments, the pathogenic bacterial strain is *B. fragilis*, *E. faecalis*, and/or *C. perfringens*. In some embodiments, the pathogenic bacterial strain is a strain of *B. fragilis* expressing the BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens* expressing the collagenase ColA. In some embodiments, the pathogenic bacterial strain is a strain of *B. fragilis* expressing the BFT toxin. In some embodiments, the subject is colonized by *B. fragilis*, *E. faecalis*, or *C. perfringens*. In some embodiments, the subject is colonized by *B. fragilis*, *E. faecalis*, and *C. perfringens*. In some embodiments, the subject is colonized by *B. fragilis* and *E. faecalis*. In some embodiments, the subject is colonized by *B. fragilis* and *C. perfringens*. In some embodiments, the subject is colonized by *E. faecalis* and *C. perfringens*. In some embodiments, the subject is colonized by *B. fragilis*. In some embodiments, the subject is colonized by an enterotoxigenic strain of *B. fragilis* (ETBF). In some embodiments, a subject is colonized with more than one strain of ETBF. In some embodiments, a subject that is colonized with ETBF is also be colonized with one or more strains of NTBF. In some embodiments, colonization is by one or more strain of ETBF. In some embodiments, the subject is colonized by *E. faecalis*. In some embodiments, the subject is colonized by *C. perfringens*.

**[0166]** In some embodiments, the method for treating or preventing a disease or disorder in a subject comprises administering to the subject a therapeutically effective amount of a compound of the present disclosure (e.g., a compound of Formula I, Formula IA, Formula, IB, Formula IB-1, Formula IC, Formula IC-1, Formula II, Formula III, Formula IV, Formula IVA, Formula IVB, Formula V, or Formula VA) that diminishes the pathogenic effects of a strain of *B. fragilis* expressing the BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens* expressing the collagenase ColA.

**[0167]** In some embodiments, the method for treating or preventing a disease or disorder in a subject comprises administering to the subject a compound that binds to and/or inhibits the activity of one or more of BFT, ColA, and GelE. In some embodiments, the compound binds to BFT, ColA, and/or GelE with an inhibition constant in the range of about  $10^{-5}$  to about  $10^{-13}$  M, e.g., about  $10^{-5}$ , about  $10^{-6}$ , about  $10^{-7}$ , about  $10^{-8}$ , about  $10^{-9}$ , about  $10^{-10}$ , about  $10^{-11}$ , about  $10^{-12}$ , about  $10^{-13}$  M. In some embodiments, the method for treating or preventing a disease or disorder in a subject comprises administering to the subject an inhibitor of BFT, ColA, and/or GelE or a pharmaceutical composition thereof. In some embodiments, the BFT comprises the amino acid sequence of any one of SEQ ID NO: 2-4. In some embodiments, the BFT comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, or at least 98% identical to any one of SEQ ID NO: 2-4. In some embodiments, the BFT comprises an amino acid sequence that is at least 98% identical to any one of SEQ ID NO: 2-4. In some embodiments, the GelE comprises the amino acid sequence of SEQ ID NO: 6. In some embodiments, the GelE comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, or at least 98% identical to SEQ ID NO: 6. In some embodiments, the GelE comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 6. In some embodiments, the ColA comprises the amino acid sequence of SEQ ID NO: 8. In some embodiments, the ColA comprises an amino acid sequence that is at least 95%, at least 96%, at least 97%, or at least 98% identical to SEQ ID NO: 8. In some embodiments, the ColA comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 8.

**[0168]** In some embodiments of the disclosed methods, administering the compound reduces and/or eliminates the activity of at least one of BFT, ColA and/or GelE. In some embodiments of the disclosed methods, administering the compound reduces the activity of at least one of BFT, ColA and/or GelE. In some embodiments of the disclosed methods, administering the compound eliminates the activity of at least one of BFT, ColA and/or GelE. In some embodiments, administering the compound substantially eliminates the activity of at least one of BFT, ColA and/or GelE. In some embodiments, administering the compound completely eliminates the activity of at least one of BFT, ColA and/or GelE. In some embodiments, administering the compound (e.g., an inhibitor of BFT, ColA, and/or GelE) reduces the number of pathogenic bacteria in the subject. In

some embodiments, administering the compound eliminates the infection caused by the pathogenic bacteria in the subject. In some embodiments, the pathogenic bacteria is one or more of *B. fragilis*, *E. faecalis*, and *C. perfringens*.

**[0169]** In some embodiments, the disease or disorder is an inflammatory bowel disease or disorder, such as Crohn's disease or ulcerative colitis. In some embodiments, the disease or disorder is a diarrheal disease, such as short duration watery diarrhea (e.g., due to cholera), short duration bloody diarrhea (e.g., dysentery), and persistent diarrhea (e.g., lasting more than 14 days). In some embodiments, the disease is cancer. In some embodiments, the cancer is a gastrointestinal (GI) cancer. The GI cancer may be, for example, esophageal cancer, gallbladder cancer, liver cancer, pancreatic cancer, stomach cancer, cancer of the small intestine, colorectal cancer, and anal cancer. In some embodiments, the cancer is colorectal cancer, such as adenocarcinoma, gastrointestinal stromal tumors (GIST), colorectal lymphoma, carcinoids, Turcot Syndrome, Peutz-Jeghers Syndrome (PJS), Familial Colorectal Cancer (FCC), or Juvenile Polyposis Coli. The cancer may be stage I, stage II, stage III, or stage IV (i.e., metastatic).

**[0170]** In some embodiments, the subject has (or is suspected of having) one or more diseases or disorders. In some embodiments, the subject has (or is suspected of having) an inflammatory bowel disease or disorder, such as Crohn's disease or ulcerative colitis. In some embodiments, the subject has (or is suspected of having) a diarrheal disease, such as short duration watery diarrhea (e.g., due to cholera), short duration bloody diarrhea (e.g., dysentery), and persistent diarrhea (e.g., lasting more than 14 days). In some embodiments, the subject has a gastrointestinal (GI) cancer. The GI cancer may be, for example, esophageal cancer, gallbladder cancer, liver cancer, pancreatic cancer, stomach cancer, cancer of the small intestine, colorectal cancer, and anal cancer. In some embodiments, the subject has colorectal cancer, such as adenocarcinoma, gastrointestinal stromal tumors (GIST), colorectal lymphoma, carcinoids, Turcot Syndrome, Peutz-Jeghers Syndrome (PJS), Familial Colorectal Cancer (FCC), or Juvenile Polyposis Coli. The cancer may be stage I, stage II, stage III, or stage IV (i.e., metastatic).

**[0171]** In some embodiments, the subject is a mammal, such as a primate, ungulate (e.g., cow, pig, horse), domestic pet or domesticated mammal. In some cases, the subject is a mammal selected from a rabbit, pig, horse, sheep, cow, cat or dog. In some embodiments, the subject is a human. The subject may be a male, or a female. In some embodiments, the subject is greater than about 18 years old, greater than about 25 years old, greater than about 35 years old, greater than about 45 years old, greater than about 55 years old, greater than about 65 years old, greater than about 75 years old, or greater than about 85 years old. In some embodiments, the subject is less than about 18 years old, less than about 16 years old, less than about 14 years old, less than about 12 years old, less than about 10 years old, less than about 8 years old, less than about 6 years old, less than about 5 years old, less than about 4 years old, less than about 3 years old, less than about 2 years old, less than about 1 year old, or less than about 6 months old. In some embodiments, the subject is greater than or equal to 18 years old. In some embodiments, the subject is less than 18 years old.

**[0172]** In some embodiments of the disclosed methods, the compound or pharmaceutical composition is administered to the subject orally, parenterally, sublingually, transdermally, rectally, transmucosally, topically, via inhalation, via buccal administration, intrapleurally, intravenously, intraarterially, intragastrically, nasally, intraperitoneally, subcutaneously, intramuscularly, intranasally, intrathecally, and intraarticularly or combinations thereof. In some embodiments, the compound is administered orally to the subject. In some embodiments, the compound is administered in a tablet or a capsule. In some embodiments, the tablet or capsule comprises a pharmaceutically acceptable carrier or excipient. In some embodiments, the compound is administered as a liquid formulation. In some embodiments, the liquid formulation comprises a pharmaceutically acceptable carrier or excipient. In some embodiments, the compound is administered intravenously to the subject.

**[0173]** The pharmaceutical compositions described herein may be administered at a therapeutically-effective dose. As used herein, "therapeutically-effective dose" means a dose sufficient to achieve the intended therapeutic purpose, such as, to alleviate a sign or symptom of a disease or disorder in a patient. A therapeutically effective amount of compound in this invention will vary with the particular goal to be achieved, the age and

physical condition of the patient being treated, the severity of the underlying disease, the duration of treatment, the nature of concurrent therapy and the specific compound employed. For example, a therapeutically effective amount of a compound of the invention administered to a child or a neonate will be reduced proportionately in accordance with sound medical judgement. The effective amount of a compound of the invention will thus be the minimum amount which will provide the desired effect.

**[0174]** The amount of compound administered will depend upon a variety of factors, including, for example, the particular indication being treated, the route of administration, whether the desired benefit is prophylactic or therapeutic, the severity of the indication being treated and the age and weight of the patient, the bioavailability of the particular active compound, and the like. Determination of an effective dosage is well within the capabilities of those skilled in the art.

**[0175]** Effective dosages can be estimated initially from *in vitro* assays. For example, an initial dosage for use in animals can be formulated to achieve a circulating blood or serum concentration of active compound that is at or above an IC<sub>50</sub> of the particular compound as measured in an *in vitro* assay. Calculating dosages to achieve such circulating blood, serum, or intestinal concentrations taking into account the bioavailability of the particular compound is well within the capabilities of skilled artisans. For guidance, see Fingl & Woodbury, "General Principles," In: *Goodman and Gilman's The Pharmaceutical Basis of Therapeutics*, Chapter 1, pp. 1-46, latest edition, Pergamon Press, and the references cited therein, which are incorporated herein by reference.

**[0176]** Initial dosages also can be estimated from *in vivo* data, such as animal models. Animal models useful for testing the efficacy of compounds to treat or prevent the various diseases described above are well-known in the art.

**[0177]** Dosage amounts will typically be in the range of from about 0.0001 or 0.001 or 0.01 mg/kg/day to about 100 mg/kg/day, but can be higher or lower, depending upon, among other factors, the activity of the compound, its bioavailability, the mode of administration, and various factors discussed above. In some embodiments, a dose of the compound administered to the subject is from about 0.001 to about 1000 mg/kg of body weight per day, e.g., about 0.001 mg/kg of body weight per day, about 0.01 mg/kg of body weight per day, about 0.1 mg/kg of body weight per day, about 1 mg/kg of body

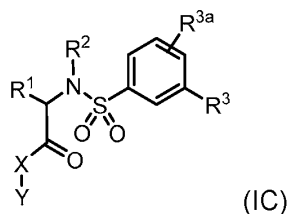
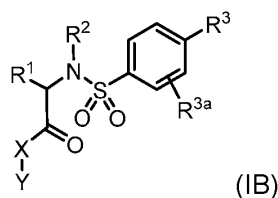
weight per day, about 10 mg/kg of body weight per day, about 100 mg/kg of body weight per day, or about 1000 mg/kg of body weight today, including all ranges and values therebetween. Dosage amount and interval can be adjusted individually to provide plasma levels of the compound(s) which are sufficient to maintain therapeutic or prophylactic effect. In cases of local administration or selective uptake, such as local topical administration, the effective local concentration of active compound(s) cannot be related to plasma concentration. Skilled artisans will be able to optimize effective local dosages without undue experimentation.

**[0178]** The inhibitor (or a pharmaceutical composition comprising the same) can be administered once per day, once per week, or multiple times per day (e.g., bid, tid, qid, etc.) or week. Administration frequency may depend upon, among other things, the indication being treated and the judgment of the prescribing physician. A treatment of a subject with a therapeutically effective amount of a compound can include a single treatment or, preferably, can include a series of treatments. In another example, a subject may be treated daily for several years in the setting of a chronic condition or illness. It will also be appreciated that the effective dosage used for treatment may increase or decrease over the course of a particular treatment.

**[0179]** The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this disclosure contains references to specific embodiments, it is apparent that other embodiments and variations of this disclosure may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

**[0180] Numbered Embodiments of the Disclosure:**

**[0181]** 1. A compound of Formula IB or IC:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{--NH--}$ ,  $\text{--N(C}_{1-5}\text{ alkyl)--}$ ,  $\text{--N(CH}_2\text{CF}_3\text{)--}$ , or  $\text{--O--}$ ;

Y is H,  $\text{--OH}$ ,  $\text{--OCH}_3$ ,  $\text{--OCH}_2\text{CH}_3$ ,  $\text{--NH}_2$ ,  $\text{--NH(CH}_3\text{)}$ ,  $\text{--NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{--NH(CH}_2\text{CF}_3\text{)}$ ;

wherein:

when X is  $\text{--NH--}$ ,  $\text{--N(C}_{1-5}\text{ alkyl)--}$ , or  $\text{--N(CH}_2\text{CF}_3\text{)--}$ , Y is  $\text{--OH}$ ,  $\text{--OCH}_3$ ,  $\text{--OCH}_2\text{CH}_3$ ,  $\text{--NH}_2$ ,  $\text{--NH(CH}_3\text{)}$ ,  $\text{--NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{--NH(CH}_2\text{CF}_3\text{)}$ ; and

when X is  $\text{--O--}$ , Y is H;

$\text{R}^1$  is H, alkyl, haloalkyl,  $\text{--alkylene-OH}$ ,  $\text{--alkylene-NH}_2$ ,  $\text{--alkylene-C(=O)NH}_2$ , heteroaralkyl, aryl, aralkyl,  $\text{--alkylene-S-alkyl}$ ,  $\text{--alkylene-S-haloalkyl}$ ,  $\text{--alkylene-S-aralkyl}$ , or  $\text{--alkylene-S-heteroaralkyl}$ ; wherein  $\text{R}^1$  is optionally substituted with one or more groups selected from  $\text{--OH}$ , halogen,  $\text{--CHF}_2$ ,  $\text{--CH}_2\text{F}$ , or  $\text{--CF}_3$ ;

$\text{R}^2$  is H,  $\text{--CH}_2\text{--aryl}$ ,  $\text{--CH}_2\text{--alkyl}$ ,  $\text{--CH(Me)--alkyl}$ ,  $\text{--CH}_2\text{--heterocyclyl}$ ,  $\text{--CH}_{2n}\text{--heteroaryl}$ , or  $\text{--CH}_2\text{--haloalkyl}$ ;

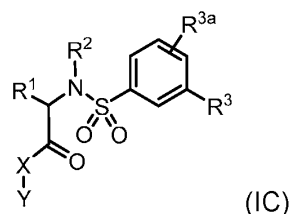
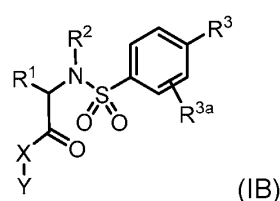
$\text{R}^3$  is  $\text{--OH}$ , alkoxy,  $\text{--O-alkylene-NR}^5\text{R}^6$ , alkyl,  $\text{--S-alkyl}$ ,  $\text{--NH-alkyl}$ ,  $\text{--N(CH}_3\text{)--alkyl}$ ,  $\text{--N(H)aralkyl}$ ,  $\text{--N(H)C(O)-alkylene-NR}^5\text{R}^6$ ,  $\text{--O-haloalkyl}$ ,  $\text{--O-aryl}$ ,  $\text{--O-heteroaryl}$ , or  $\text{--O-aralkyl}$ , each of which is optionally substituted;

$\text{R}^{3a}$  is H or halogen;

$\text{R}^5$  is H,  $\text{--C(O)alkyl}$ ,  $\text{--C(O)aryl}$ ,  $\text{--C(O)heteroaryl}$ , or  $\text{--C(O)aralkyl}$ ; and

$\text{R}^6$  is H, alkyl, or aryl.

**[0182]** 1a. A compound of Formula IB or IC:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{--NH--}$ ,  $\text{--N(C}_{1-5}\text{ alkyl)--}$ ,  $\text{--N(CH}_2\text{CF}_3\text{)--}$ , or  $\text{--O--}$ ;

Y is H,  $\text{--OH}$ ,  $\text{--OCH}_3$ ,  $\text{--OCH}_2\text{CH}_3$ ,  $\text{--NH}_2$ ,  $\text{--NH(CH}_3\text{)}$ ,  $\text{--NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{--NH(CH}_2\text{CF}_3\text{)}$ ;

wherein:

when X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$ , Y is  $\text{—OH}$ ,  $\text{—OCH}_3$ ,  $\text{—OCH}_2\text{CH}_3$ ,  $\text{—NH}_2$ ,  $\text{—NH(CH}_3\text{)}$ ,  $\text{—NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ ; and

when X is  $\text{—O—}$ , Y is H;

$\text{R}^1$  is H, alkyl, haloalkyl,  $\text{—alkylene—OH}$ ,  $\text{—alkylene—NH}_2$ ,  $\text{—alkylene—C(=O)NH}_2$ , heteroaralkyl, aryl, aralkyl,  $\text{—alkylene—S—alkyl}$ ,  $\text{—alkylene—S—haloalkyl}$ ,  $\text{—alkylene—S—aralkyl}$ , or  $\text{—alkylene—S—heteroaralkyl}$ ; wherein  $\text{R}^1$  is optionally substituted with one or more groups selected from  $\text{—OH}$ , halogen,  $\text{—CHF}_2$ ,  $\text{—CH}_2\text{F}$ , or  $\text{—CF}_3$ ;

$\text{R}^2$  is H,  $\text{—(CH}_2\text{)}_n\text{—aryl}$ ,  $\text{—CH}_2\text{—alkyl}$ ,  $\text{—CH(Me)—alkyl}$ ,  $\text{—CH}_2\text{—heterocyclyl}$ ,  $\text{—(CH}_2\text{)}_n\text{—heteroaryl}$ , or  $\text{—CH}_2\text{—haloalkyl}$ ;

$\text{R}^3$  is  $\text{—OH}$ , alkoxy,  $\text{—O—alkylene—NR}^5\text{R}^6$ , alkyl,  $\text{—S—alkyl}$ ,  $\text{—NH—alkyl}$ ,  $\text{—N(CH}_3\text{)—alkyl}$ ,  $\text{—N(H)—aralkyl}$ ,  $\text{—N(H)C(O)—alkylene—NR}^5\text{R}^6$ ,  $\text{—O—haloalkyl}$ ,  $\text{—O—aryl}$ ,  $\text{—O—heteroaryl}$ , or  $\text{—O—aralkyl}$ , each of which is optionally substituted;

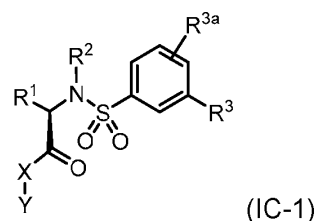
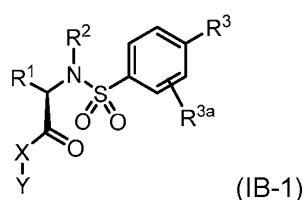
$\text{R}^{3a}$  is H or halogen;

$\text{R}^5$  is H,  $\text{—C(O)—alkyl}$ ,  $\text{—C(O)—aryl}$ ,  $\text{—C(O)—heteroaryl}$ , or  $\text{—C(O)—aralkyl}$ ;

$\text{R}^6$  is H, alkyl, or aryl; and

n is an integer from 0-3.

**[0183]** 2. The compound of embodiment 1 or 1a, wherein the compound of Formula IB has the structure of Formula IB-1 or IC-1:



or a stereoisomer or a pharmaceutically acceptable salt thereof.

**[0184]** 3. The compound of embodiment 1, 1a, or 2, wherein X is  $\text{—NH—}$  or  $\text{—N(C}_{1-5}\text{ alkyl)—}$ .

**[0185]** 4. The compound of any one of embodiments 1-3, wherein the  $\text{C}_{1-5}$  alkyl is methyl or ethyl.

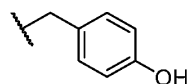
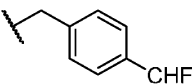
**[0186]** 5. The compound of any one of embodiments 1-3, wherein X is  $\text{—NH—}$

**[0187]** 6. The compound of any one of embodiments 1-5, wherein Y is -OH, -OCH<sub>3</sub>, or -OCH<sub>2</sub>CH<sub>3</sub>.

**[0188]** 7. The compound of any one of embodiments 1-6, wherein Y is -OH.

**[0189]** 8. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is -C<sub>1</sub>-C<sub>6</sub> alkyl, -C<sub>1</sub>-C<sub>6</sub> alkyl-OH, -(C<sub>1</sub>-C<sub>3</sub> alkylene)-S-(C<sub>1</sub>-C<sub>3</sub> alkyl), -(C<sub>1</sub>-C<sub>3</sub> alkylene)-S-(C<sub>1</sub>-C<sub>3</sub> haloalkyl), -(C<sub>1</sub>-C<sub>3</sub> alkylene)-SCH<sub>2</sub>-heteroaryl, -CH<sub>2</sub>-phenyl, -CH<sub>2</sub>-heteroaryl, or -CH<sub>2</sub>C(=O)NH<sub>2</sub>, wherein phenyl is optionally substituted with one or more groups selected from -OH, -OMe, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>.

**[0190]** 9. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is -

CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH(OH)CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>, , -CH<sub>2</sub>-phenyl, -CH<sub>2</sub>-(3-indolyl), , -CH<sub>2</sub>C(=O)NH<sub>2</sub>, -CH(CH<sub>3</sub>)SCH<sub>2</sub>CH<sub>3</sub>, -CH(CH<sub>3</sub>)SCH<sub>2</sub>-(3-pyridyl), -CH(CH<sub>3</sub>)SCH<sub>2</sub>-(4-pyridyl), or -CH(CH<sub>3</sub>)SCH<sub>2</sub>CF<sub>3</sub>.

**[0191]** 10. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is H, alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-S-alkyl, heteroaralkyl, aryl, or aralkyl.

**[0192]** 11. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is H, alkyl, -alkylene-OH, alkylene-O-alkyl, heteroaralkyl, aryl, or aralkyl.

**[0193]** 12. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is H, alkyl, -alkylene-OH, alkylene-O-alkyl.

**[0194]** 13. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is H or alkyl.

**[0195]** 14. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is alkyl.

**[0196]** 15. The compound of any one of embodiments 1-7, wherein R<sup>1</sup> is H.

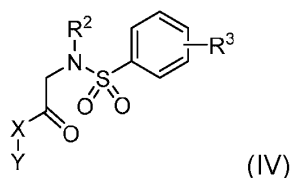
**[0197]** 16. The compound of any one of embodiments 10-14, wherein the alkyl is a C<sub>1-6</sub> alkyl.

- [0198]** 17. The compound of any one of embodiments 10-14, wherein the alkyl is an isobutyl or ethyl group.
- [0199]** 17a. The compound of any one of embodiments 10-14, wherein the alkyl is an ethyl group.
- [0200]** 18. The compound of any one of embodiments 1-17a, wherein  $R^2$  is -CH<sub>2</sub>-alkyl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.
- [0201]** 18a. The compound of any one of embodiments 1a-17a, wherein  $R^2$  is -CH<sub>2</sub>-alkyl, -(CH<sub>2</sub>)<sub>n</sub>-aryl, or -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl.
- [0202]** 19. The compound of any one of embodiments 1a-18, wherein  $R^2$  is -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl.
- [0203]** 20. The compound of any one of embodiments 18, 18a, and 19, wherein the heteroaryl is a 5- or 6-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S.
- [0204]** 21. The compound of any one of embodiments 18-20, wherein the heteroaryl is a 5-membered heteroaryl having 1, 2, or 3 heteroatoms selected from the group consisting of N, O, and S.
- [0205]** 22. The compound of any one of embodiments 18, 18a, and 19, wherein the heteroaryl is pyridyl, thiazoyl, oxazolyl, or indolyl.
- [0206]** 23. The compound of any one of embodiments 18, 18a, and 19, wherein the heteroaryl is 3-pyridyl or 5-indolyl.
- [0207]** 23a. The compound of any one of embodiments 18, 18a, and 19, wherein the heteroaryl is 3-pyridyl.
- [0208]** 24. The compound of any one of embodiments 1a-23a, wherein n is 1 or 2.
- [0209]** 24a. The compound of any one of embodiments 1a-23a, wherein n is 2.

- [0210]** 25. The compound of any one of embodiments 1-24a, wherein  $R^3$  is -OH, alkoxy, -O-alkylene- $NR^5R^6$ , alkyl or -N(H)C(O)-alkylene- $NR^5R^6$ , wherein the alkylene is optionally substituted with F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.
- [0211]** 26. The compound of any one of embodiments 1-25, wherein  $R^3$  is -OH or alkoxy.
- [0212]** 26a. The compound of any one of embodiments 1-26, wherein  $R^3$  is -OH.
- [0213]** 27. The compound of any one of embodiments 1-26, wherein the alkoxy is -OCH<sub>3</sub>.
- [0214]** 28. The compound of any one of embodiments 1-25, wherein the alkylene is a C<sub>1-3</sub> alkylene.
- [0215]** 29. The compound of any one of embodiments 1-25, wherein the alkylene is a methylene or ethylene.
- [0216]** 30. The compound of any one of embodiments 1-29, wherein  $R^5$  is H, aralkyl, or heteroaralkyl.
- [0217]** 30a. The compound of embodiment 30, wherein the aralkyl is -CH<sub>2</sub>aryl.
- [0218]** 30b. The compound of embodiment 30 or 30a, wherein the aralkyl is -CH<sub>2</sub>Ph.
- [0219]** 30c. The compound of any one of embodiments 30-30b, wherein the heteroaralkyl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl.
- [0220]** 31. The compound of any one of embodiments 1-30c, wherein  $R^6$  is H or alkyl.
- [0221]** 31a. The compound of embodiment 31, wherein the alkyl is aralkyl or heteroaralkyl.
- [0222]** 31b. The compound of embodiment 31a, wherein the aralkyl is -CH<sub>2</sub>aryl.
- [0223]** 31c. The compound of embodiment 31a or 31b, wherein the aralkyl is -CH<sub>2</sub>Ph.

**[0224]** 31d. The compound of any one of embodiments 31a-31c, wherein the heteroaralkyl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl.

**[0225]** 32. A compound of Formula IV:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is -NH-, -N(C<sub>1-5</sub> alkyl)-, or -N(CH<sub>2</sub>CF<sub>3</sub>)-, Y is -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>); and

when X is -O-, Y is H;

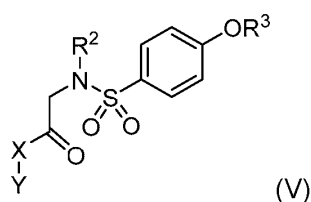
R<sup>2</sup> is alkyl;

R<sup>3</sup> is -OH, alkoxy, -O-alkylene-NR<sup>5</sup>R<sup>6</sup>, alkyl, -S-alkyl, -NH-alkyl, -N(CH<sub>3</sub>)-alkyl, -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>, -O-haloalkyl, -O-aryl, or -O-heteroaryl, each of which is optionally substituted;

R<sup>5</sup> is H, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl; and

R<sup>6</sup> is H, alkyl, or aryl.

**[0226]** 32a. A compound of Formula V:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-, -N(C<sub>1-5</sub> alkyl)-, -N(CH<sub>2</sub>CF<sub>3</sub>)-, or -O-;

Y is H, -OH, -OCH<sub>3</sub>, -OCH<sub>2</sub>CH<sub>3</sub>, -NH<sub>2</sub>, -NH(CH<sub>3</sub>), -NH(CH<sub>2</sub>CH<sub>3</sub>), or -NH(CH<sub>2</sub>CF<sub>3</sub>);

wherein:

when X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$ , Y is  $\text{—OH}$ ,  $\text{—OCH}_3$ ,  $\text{—OCH}_2\text{CH}_3$ ,  $\text{—NH}_2$ ,  $\text{—NH(CH}_3\text{)}$ ,  $\text{—NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ ; and

when X is  $\text{—O—}$ , Y is H;

$\text{R}^2$  is H,  $\text{—CH}_2\text{—aryl}$ ,  $\text{—CH}_2\text{—alkyl}$ ,  $\text{—CH(Me)—alkyl}$ ,  $\text{—CH}_2\text{—heteroaryl}$ , or  $\text{—CH}_2\text{—haloalkyl}$ ;

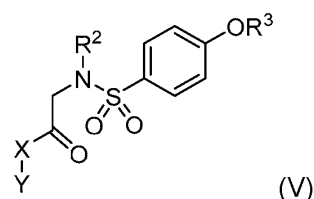
and

$\text{R}^3$  is H, alkyl,  $\text{—alkylene—NR}^5\text{R}^6$ , haloalkyl, aryl, aralkyl, or heteroaryl, each of which is optionally substituted;

$\text{R}^5$  is H, alkyl, aralkyl, heteroaralkyl,  $\text{—C(O)alkyl}$ ,  $\text{—C(O)aryl}$ ,  $\text{—C(O)heteroaryl}$ , or  $\text{—C(O)aralkyl}$ ; and

$\text{R}^6$  is H, alkyl, or aryl.

**[0227]** 32b. A compound of Formula V:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ ,  $\text{—N(CH}_2\text{CF}_3\text{)—}$ , or  $\text{—O—}$ ;

Y is H,  $\text{—OH}$ ,  $\text{—OCH}_3$ ,  $\text{—OCH}_2\text{CH}_3$ ,  $\text{—NH}_2$ ,  $\text{—NH(CH}_3\text{)}$ ,  $\text{—NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ ;

wherein:

when X is  $\text{—NH—}$ ,  $\text{—N(C}_{1-5}\text{ alkyl)—}$ , or  $\text{—N(CH}_2\text{CF}_3\text{)—}$ , Y is  $\text{—OH}$ ,  $\text{—OCH}_3$ ,  $\text{—OCH}_2\text{CH}_3$ ,  $\text{—NH}_2$ ,  $\text{—NH(CH}_3\text{)}$ ,  $\text{—NH(CH}_2\text{CH}_3\text{)}$ , or  $\text{—NH(CH}_2\text{CF}_3\text{)}$ ; and

when X is  $\text{—O—}$ , Y is H;

$\text{R}^2$  is H,  $\text{—(CH}_2\text{)}_n\text{—aryl}$ ,  $\text{—CH}_2\text{—alkyl}$ ,  $\text{—CH(Me)—alkyl}$ ,  $\text{—(CH}_2\text{)}_n\text{—heteroaryl}$ , or  $\text{—CH}_2\text{—haloalkyl}$ ; and

$\text{R}^3$  is H, alkyl,  $\text{—alkylene—NR}^5\text{R}^6$ , haloalkyl, aryl, aralkyl, or heteroaryl, each of which is optionally substituted;

$\text{R}^5$  is H, alkyl, aralkyl, heteroaralkyl,  $\text{—C(O)alkyl}$ ,  $\text{—C(O)aryl}$ ,  $\text{—C(O)heteroaryl}$ , or  $\text{—C(O)aralkyl}$ ;

R<sup>6</sup> is H, alkyl, or aryl; and

n is an integer from 0-3.

**[0228]** 33. The compound of any one of embodiments 32, 32a, or 32b, wherein X is -NH- or -N(C<sub>1-5</sub> alkyl)-.

**[0229]** 34. The compound of any one of embodiments 32, 32b, and 33, wherein the C<sub>1-5</sub> alkyl is methyl or ethyl.

**[0230]** 35. The compound of any one of embodiments 32-34, wherein X is -NH-.

**[0231]** 36. The compound of any one of embodiments 32-35, wherein Y is -OH, -OCH<sub>3</sub>, or -OCH<sub>2</sub>CH<sub>3</sub>.

**[0232]** 37. The compound of any one of embodiments 32-36, wherein Y is -OH.

**[0233]** 37a. The compound of any one of embodiments 32-37, wherein R<sup>2</sup> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>), -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or -CH<sub>2</sub>CHF<sub>2</sub>.

**[0234]** 37b. The compound of embodiment 37a, wherein R<sup>2</sup> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>.

**[0235]** 37c. The compound of any one of embodiments 32a-37, wherein R<sup>2</sup> is -CH<sub>2</sub>-aryl, -CH<sub>2</sub>-alkyl, -or -CH<sub>2</sub>-heteroaryl.

**[0236]** 37d. The compound of any one of embodiments 32a-37c, wherein R<sup>2</sup> is -CH<sub>2</sub>-Ph, -CH<sub>2</sub>-CH(CH<sub>3</sub>)<sub>2</sub>, -or -CH<sub>2</sub>-(3-pyridyl).

**[0237]** 38. The compound of any one of embodiments 32-37, wherein R<sup>3</sup> is -OH, alkoxy, -O-haloalkyl, -O-aralkyl, -O-heteroaralkyl, -O-alkylene-NR<sup>5</sup>R<sup>6</sup>, alkyl or -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>, wherein the alkylene is optionally substituted with F, oxo, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

**[0238]** 39. The compound of any one of embodiments 32-38, wherein R<sup>3</sup> is -OH, alkoxy, or -O-alkylene-NR<sup>5</sup>R<sup>6</sup>.

**[0239]** 40. The compound of any one of embodiments 32-39, wherein the alkoxy is  $-\text{OCH}_3$ .

**[0240]** 41. The compound of any one of embodiments 32-38, wherein the alkylene is a  $\text{C}_{1-3}$  alkylene.

**[0241]** 42. The compound of any one of embodiments 32-38, wherein the alkylene is a methylene or ethylene.

**[0242]** 42a. The compound of embodiment 39, wherein the  $-\text{O-alkylene-NR}^5\text{R}^6$  is  $-\text{O-CH}_2\text{-C(O)-NR}^5\text{R}^6$ .

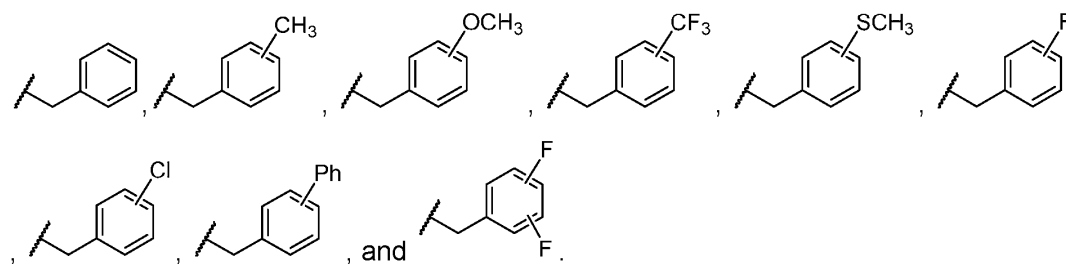
**[0243]** 43. The compound of any one of embodiments 32-42a, wherein  $\text{R}^5$  is H or aralkyl or heteroaralkyl.

**[0244]** 43a. The compound of embodiment 43, wherein the aralkyl is  $-\text{CH}_2\text{aryl}$ .

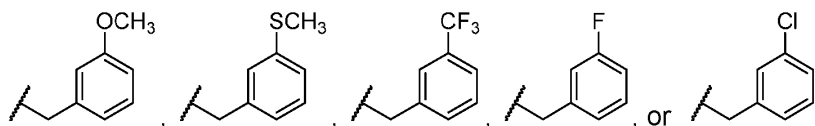
**[0245]** 43b. The compound of embodiment 43 or 43a, wherein the aralkyl is  $-\text{CH}_2\text{Ph}$ .

**[0246]** 43c. The compound of embodiment 43b, wherein the Ph is optionally substituted with one or more halogen, alkyl, haloalkyl, alkoxy, thioalkyl, aryl, heteroaryl or combinations thereof.

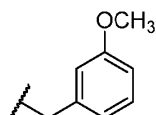
**[0247]** 43d. The compound of embodiment 43b or 43c, wherein aralkyl is selected from the group consisting of:



**[0248]** 43e. The compound of embodiments 43b or 43c, wherein the aralkyl is

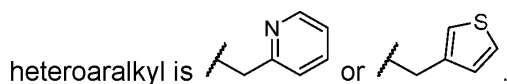


**[0249]** 43f. The compound of embodiments 43b or 43c, wherein the aralkyl is



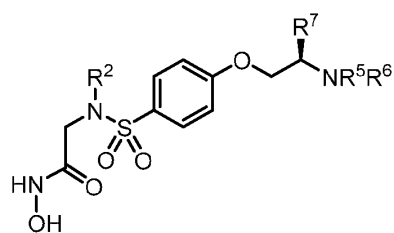
**[0250]** 43g. The compound of any one of embodiments 43-43e, wherein the heteroaralkyl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl.

**[0251]** 43h. The compound of any one of embodiments 43-43e, wherein the

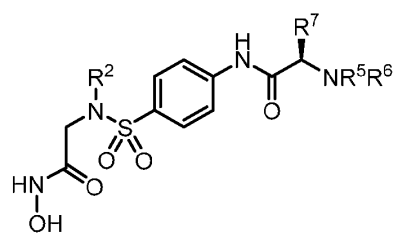


**[0252]** 44. The compound of any one of embodiments 32-43, wherein R<sup>6</sup> is H or alkyl.

**[0253]** 45. The compound of embodiment 32, wherein the compound of Formula IV has a structure according to:



(IVA)



(IVB)

or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

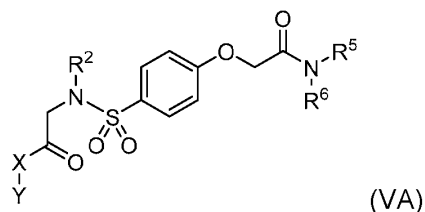
R<sup>2</sup> is alkyl;

R<sup>5</sup> is H, aralkyl or heteroaralkyl;

R<sup>6</sup> is H, alkyl, or aryl; and

R<sup>7</sup> is F, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

**[0254]** 45a. The compound of embodiment 32a, wherein the compound of Formula V has a structure according to:



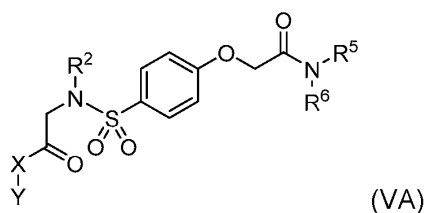
or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

$R^2$  is alkyl,  $-\text{CH}_2\text{-aryl}$ , or  $-\text{CH}_2\text{-heteroaryl}$ ;

$R^5$  is H, aralkyl or heteroaralkyl; and

$R^6$  is H, alkyl, or aryl.

**[0255]** 45b. The compound of embodiment 32b, wherein the compound of Formula V has a structure according to:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

$R^2$  is alkyl,  $-(\text{CH}_2)_n\text{-aryl}$ , or  $-(\text{CH}_2)_n\text{-heteroaryl}$ ;

$R^5$  is H, aralkyl or heteroaralkyl;

$R^6$  is H, alkyl, or aryl; and

$n$  is an integer from 0-3.

**[0256]** 46. The compound of embodiment 45, 45a or 45b, wherein  $R^2$  is a  $\text{C}_{1-5}$  alkyl.

**[0257]** 46a. The compound of embodiment 45a or 45b, wherein  $R^2$  is  $\text{C}_{1-5}$  alkyl,  $-\text{CH}_2\text{Ph}$  or  $-\text{CH}_2\text{pyridyl}$ .

**[0258]** 46b. The compound of embodiment 45b, wherein  $R^2$  is  $\text{C}_{1-5}$  alkyl,  $-\text{CH}_2\text{CH}_2\text{Ph}$  or  $-\text{CH}_2\text{CH}_2\text{pyridyl}$ .

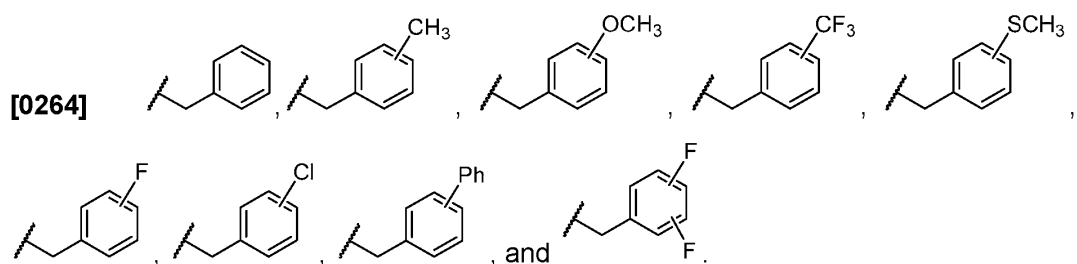
**[0259]** 46c. The compound of embodiment 45, 45a or 45b, wherein  $R^2$  is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>), -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or -CH<sub>2</sub>CHF<sub>2</sub>.

**[0260]** 46d. The compound of embodiment 45, 45a or 45b, wherein  $R^2$  is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>.

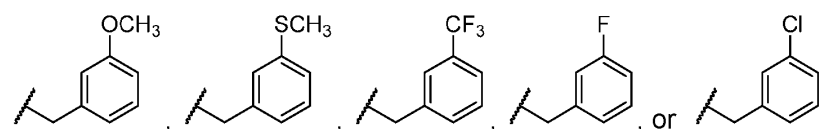
**[0261]** 47. The compound of embodiment 45, 45a or 45b, wherein  $R^2$  is Me, Et, Pr, *i*Pr, Bu, *i*Bu, or *s*Bu.

**[0262]** 47a. The compound of any one of embodiments 45a-47, wherein  $R^5$  is -CH<sub>2</sub>aryl or -CH<sub>2</sub>heteroaryl.

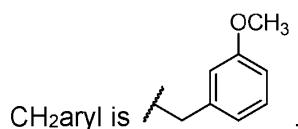
**[0263]** 47b. The compound of embodiment 47a, wherein the -CH<sub>2</sub>aryl is selected from the group consisting of:



**[0265]** 47c. The compound of embodiment 47a or 47b, wherein the -CH<sub>2</sub>aryl is

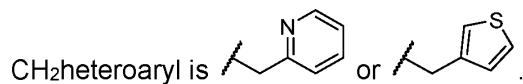


**[0266]** 47d. The compound of any one of embodiments 47a-47c, wherein the -



**[0267]** 47e. The compound of any one of embodiments 47a-47d, wherein the -CH<sub>2</sub>heteroaryl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl.

**[0268]** 47f. The compound of any one of embodiments 47a-47e, wherein the -



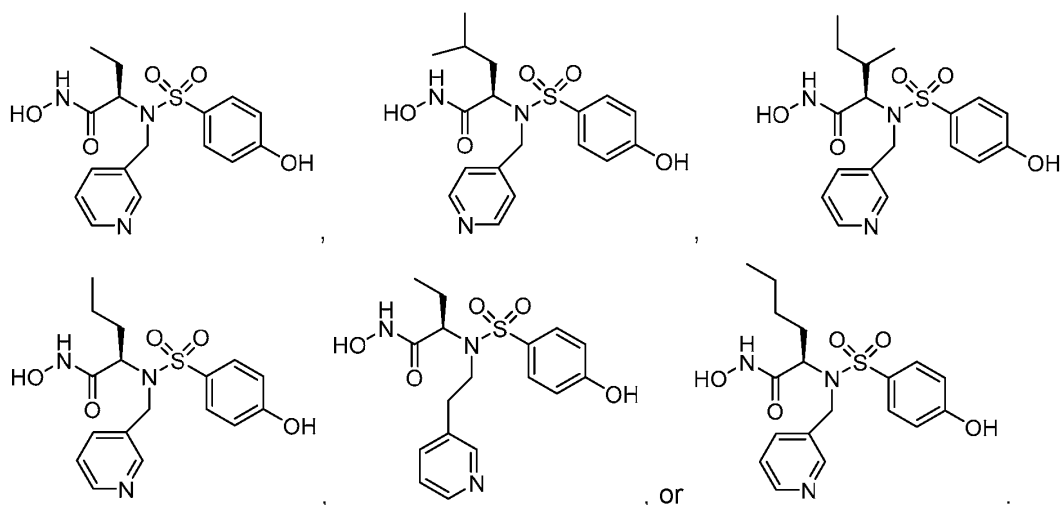
**[0269]** 47g. The compound of any one of embodiments 45-47f, wherein R<sup>6</sup> is H.

**[0270]** 47h. The compound of embodiment 45b, wherein n is 1 or 2.

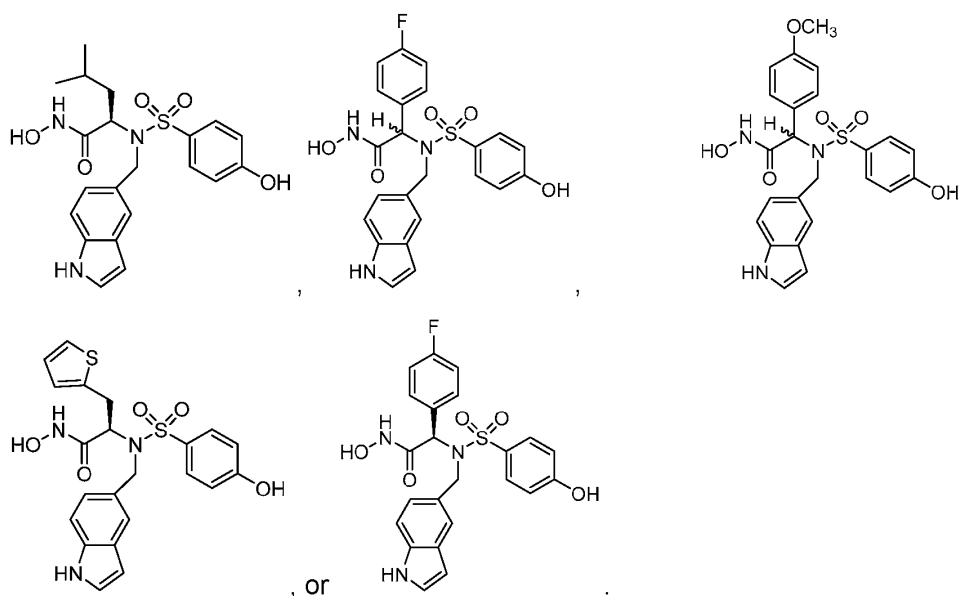
**[0271]** 47i. The compound of embodiment 45b, wherein n is 1.

**[0272]** 48. The compound of embodiment 1, having a structure according to a compound of Table 10.

**[0273]** 49. The compound of embodiment 1 or 1a, wherein the compound of Formula I has a structure according to:

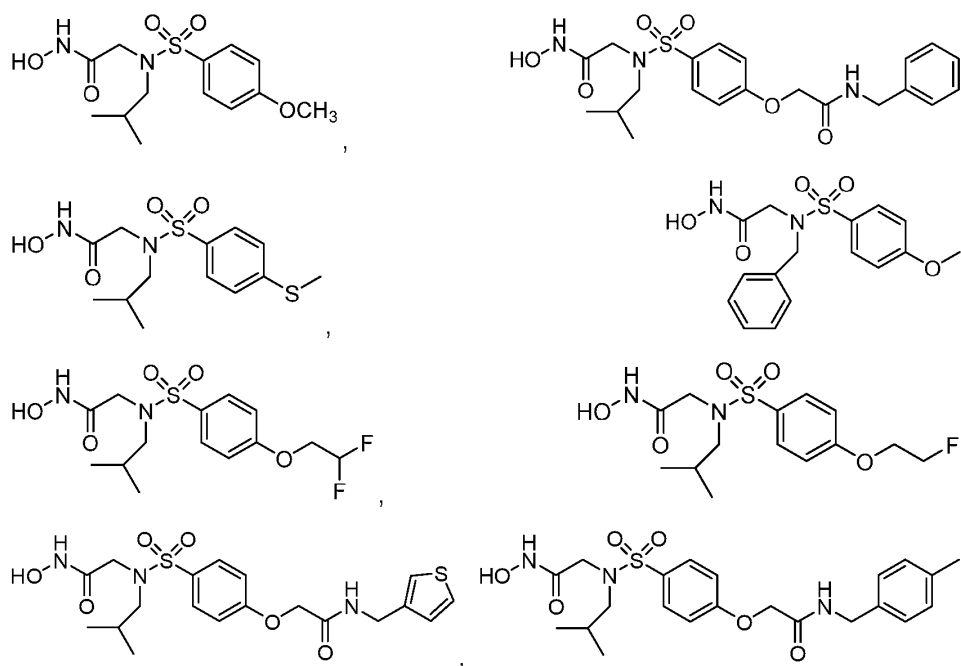


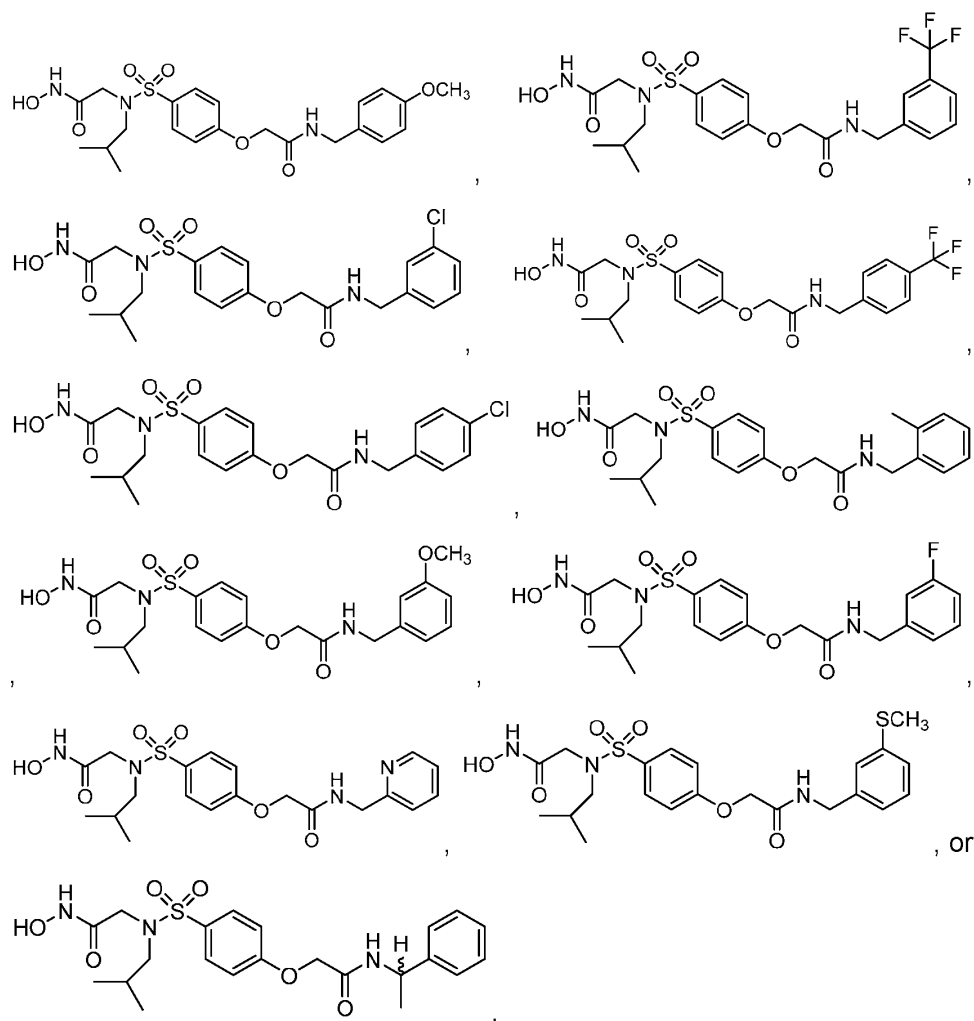
**[0274]** 49a. The compound of embodiment 1 or 1a, wherein the compound of Formula I has a structure according to:



or a stereoisomer or a pharmaceutically acceptable salt thereof.

**[0275]** 50. The compound of embodiment 32, 32a, or 32b, wherein the compound has a structure according to:





or a stereoisomer or a pharmaceutically acceptable salt thereof.

## EXAMPLES

**[0276]** The disclosure is further described in detail by reference to the following examples. These examples are provided for purposes of illustration only and are not intended to be limiting unless otherwise specified. Thus, the disclosure should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

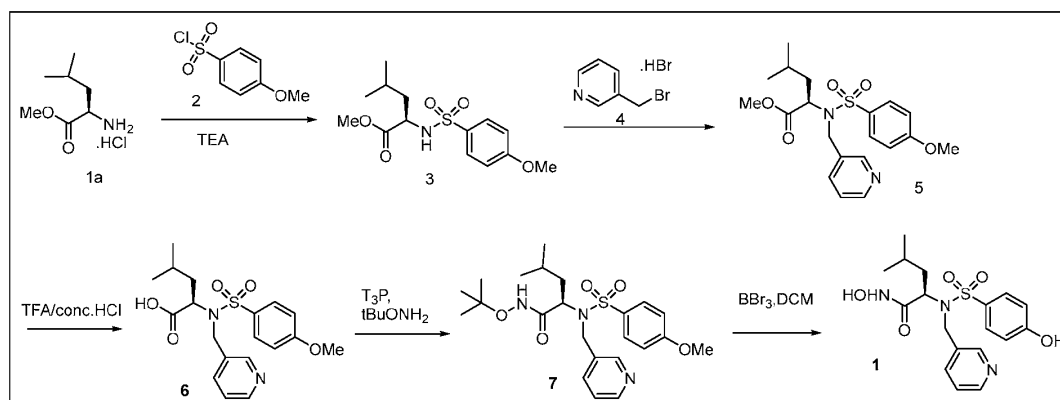
[0277] Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present disclosure and practice the claimed methods. The following working examples therefore specifically point out the preferred embodiments and are not to be construed as limiting in any way the remainder of the disclosure.

**Table 8.** Abbreviations referred to herein

| Abbreviation   | Description                               |
|----------------|-------------------------------------------|
| mmol           | Millimolar                                |
| vol            | Volume                                    |
| g              | Grams                                     |
| kg             | Kilograms                                 |
| L              | Litres                                    |
| mL             | Millilitres                               |
| °C             | Degrees celsius                           |
| TLC            | Thin layer chromatography                 |
| HPLC           | High-performance liquid chromatography    |
| LCMS           | Liquid chromatography - mass spectrometry |
| min            | Minutes                                   |
| h              | Hours                                     |
| eq             | Equivalents                               |
| RT             | Room temperature                          |
| R <sub>f</sub> | Retention factor                          |
| RM             | Reaction mixture                          |
| RP             | Reversed phase                            |
| NMR            | Nuclear magnetic resonance                |
| ppm            | Parts per million                         |

#### EXAMPLE 1

[0278] Synthesis of (R)-N-hydroxy-2-((4-hydroxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)-4-methylpentanamide (1)



**[0279] Synthesis of methyl N-((4-methoxyphenyl)sulfonyl)-D-leucinate (3):** To a stirred solution of methyl D-leucinate·HCl (1.0 g, 5.50 mmol, 1 eq) in DCM (30 mL) at 0 °C, were added triethylamine (833 mg, 8.25 mmol, 1.5 eq), and 4-methoxybenzenesulfonyl chloride (1.26 g, 6.60 mmol, 1.2 eq), under N<sub>2</sub> atmosphere. The RM was stirred at RT for 16h. After completion of the reaction, water was added and the mixture extracted with DCM (2x200 mL). The combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product purified by column chromatography over silica gel using 10% EtOAc/heptane as eluent to obtain methyl N-((4-methoxyphenyl)sulfonyl)-D-leucinate (3; 800.0 mg, 46%) as color-less liquid. TLC: 40% EtOAc/heptane (*R<sub>f</sub>*: 0.6); LCMS: 98.56%, *m/z*=316.2 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 8.12-8.18 (m, 1H), 7.67 (d, *J*=8.80 Hz, 2H), 7.09 (d, *J*=8.80 Hz, 2H), 3.83 (s, 3H), 3.70 (dt, *J*=5.93, 8.96 Hz, 1H), 3.38 (s, 3H), 1.49-1.59 (m, 1H), 1.30-1.46 (m, 2H), 0.80 (d, *J*=6.60 Hz, 3H), 0.70 (d, *J*=6.60 Hz, 3H).

**[0280] Synthesis of methyl N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (5):** To a stirred solution of methyl ((4-methoxyphenyl)sulfonyl)-D-leucinate (1.0 g, 3.18 mmol, 1 eq) in DMF (20 mL) at RT, were added K<sub>2</sub>CO<sub>3</sub> (4.40 g, 31.88 mmol, 10 eq), followed by 3-(bromomethyl)pyridine·HBr (1.20 g, 4.77 mmol, 1.50 eq), and the RM stirred at RT for 16 h. After completion of the reaction, water was added and extracted with EtOAc (2x50 mL); the organic combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using 1% MeOH/DCM as eluent to obtain methyl

N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (5; 750 mg, 58.5%) as color-less sticky liquid. TLC: 5% MeOH/ DCM ( $R_f$ : 0.2); LCMS: 95%,  $m/z$ =407.3  $[M+H]^+$ ;  $^1H$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta$  8.56 (d,  $J$ =1.83 Hz, 1H), 8.47 (dd,  $J$ =1.59, 4.77 Hz, 1H), 7.74-7.84 (m, 3H), 7.37 (ddd,  $J$ =0.67, 4.80, 7.86 Hz, 1H), 7.10-7.15 (m, 2H), 4.58-4.66 (m, 1H), 4.41-4.49 (m, 2H), 3.83-3.88 (m, 3H), 3.33 (s, 3H), 1.24-1.51 (m, 3H), 0.78 (d,  $J$ =6.48 Hz, 3H), 0.53 (d,  $J$ =6.60 Hz, 3H).

**[0281] Synthesis of N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (6):** To a stirred solution of methyl N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (1.25 g, 3.07 mmol, 1 eq; from 2 batches of 5) at 0 °C, were added TFA (10 mL), and followed by conc. HCl (12 mL); the RM was allowed to reach RT and stirred at 90°C for 13h. The reaction was monitored by TLC, after completion of the reaction was concentrated under reduced pressure. The crude product was dissolved in diethyl ether (5 mL) and stirred for 10 minutes and filtered to obtain an N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (6; 1.0 g, 83%) as brown solid. TLC: 5% MeOH/ DCM ( $R_f$ : 0.1). LCMS: 93.7%,  $m/z$ =393.2  $[M+H]^+$ ;  $^1H$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta$  12.76-13.04 (br s, 1H), 8.84-8.87 (m, 1H), 8.76-8.79 (m, 1H), 8.45 (br d,  $J$ =8.07 Hz, 1H), 7.90-7.96 (m, 1H), 7.75-7.80 (m, 2H), 7.07-7.13 (m, 2H), 4.75-4.81 (m, 1H), 4.62-4.69 (m, 1H), 4.38 (dd,  $J$ =5.26, 9.29 Hz, 1H), 3.85 (s, 3H), 1.33-1.56 (m, 3H), 0.82 (d,  $J$ =6.36 Hz, 3H), 0.59-0.62 (m, 3H).

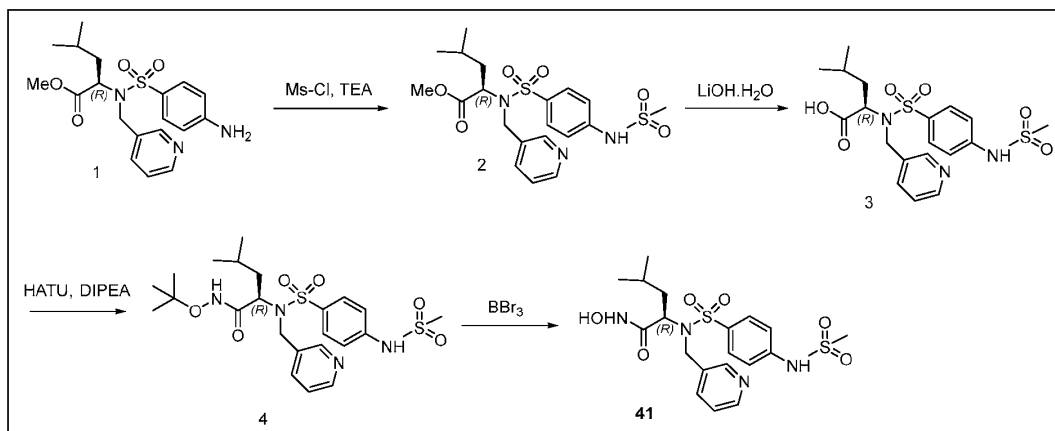
**[0282] Synthesis of (R)-N-(tert-butoxy)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido) pentanamide (7):** To a stirred solution of N-((4-methoxyphenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (1.0 g, 2.55 mmol, 1 eq) in DCM (50 mL), at 0 °C, were added TEA (1.54 g, 15.3 mmol, 6 eq), followed by propanephosphonic acid anhydride (T3P; 5 g, 7.65 mmol, 3 eq, 50% in EtOAc), and the RM stirred at 0 °C for 30 minutes. The  $NH_2OBU\cdot t.HCl$  (1.27 g, 10.20 mmol, 4 eq) was added, warmed to RT and stirred at RT for 12h. After completion of the reaction water was added and extracted with DCM (2x200 mL); the combined organic phases were dried over  $Na_2SO_4$ , filtered and concentrated under reduced pressure. The crude product purified by column chromatography over silica gel using 1% MeOH/DCM as eluent to obtain (R)-N-(tert-butoxy)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pentanamide (7; 1.0 g, 87.7%) as color-less sticky liquid. TLC: 5% MeOH/ DCM ( $R_f$ : 0.2); LCMS: 96.6%,

$m/z=464.3$   $[M+H]^+$ ;  $^1H$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta$  10.70-10.73 (m, 1H), 8.50-8.57 (m, 1H), 8.41-8.44 (m, 1H), 7.71-7.78 (m, 3H), 7.31 (dd,  $J=4.83, 7.76$  Hz, 1H), 7.06 (d,  $J=8.93$  Hz, 2H), 4.65-4.79 (m, 2H), 4.29-4.34 (m, 1H), 3.82-3.86 (m, 3H), 1.24-1.40 (m, 3H), 1.04 (s, 9H), 0.78 (d,  $J=6.24$  Hz, 3H), 0.60 (d,  $J=6.36$  Hz, 3H).

**[0283] Synthesis of (R)-N-hydroxy-2-((4-hydroxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)-4-methylpentanamide (1):** To a stirred solution of (R)-N-(tert-butoxy)-2-((4-methoxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido) pentanamide (500 mg, 1.11 mmol, 1 eq) in DCM at 0 °C, was added  $BBr_3$  (3.50 mL, 0.01 mmol, 2.5 eq, 1M in DCM), and the RM warmed to RT and stirred at 55°C for 3h. The reaction was monitored by TLC; after completion of the reaction it was concentrated under reduced pressure. The crude product was dissolved in diethyl ether (5 mL) and stirred for 10 minutes, and filtered to obtain a crude solid which was purified by prep HPLC to obtain (R)-N-hydroxy-2-((4-hydroxy-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)-4-methylpentanamide (1; 25.0 mg, 5.7%) as off white solid. TLC: 8% MeOH/ DCM ( $R_f$ : 0.3).  $^1H$  NMR (DMSO- $d_6$ , 400 MHz):  $\delta$  10.44-11.02 (m, 2H), 8.87-8.98 (m, 1H), 8.51 (d,  $J=1.71$  Hz, 1H), 8.42 (dd,  $J=1.41, 4.71$  Hz, 1H), 7.73 (br d,  $J=7.83$  Hz, 1H), 7.60 (d,  $J=8.80$  Hz, 2H), 7.30 (dd,  $J=4.83, 7.76$  Hz, 1H), 6.85 (d,  $J=8.80$  Hz, 2H), 4.67-4.74 (m, 1H), 4.54-4.60 (m, 1H), 4.18-4.24 (m, 1H), 1.37-1.45 (m, 1H), 1.21-1.30 (m, 1H), 1.11 (td,  $J=6.86, 13.30$  Hz, 1H), 0.76 (d,  $J=6.48$  Hz, 3H), 0.59-0.65 (m, 3H); LCMS: 99.34% purity,  $m/z=394.2$   $[M+H]^+$ ; (Column; Xbridge BEH-C18 (3.0X50mm, 2.5 $\mu$ m); RT:1.84 min, A: 2.5 mM Ammonium acetate, B: ACN T/B%: 0.01/5, 3/90, 5/90, 5.5/5, 6/5, 0.8 mL/min); HPLC: 99.85% purity; (Column; X-SELECT CSH C-18 (4.6 x 150 mm, 3.5  $\mu$ m); RT:4.28 min, Diluent: ACN:H<sub>2</sub>O). Chiral HPLC: >99.99% purity; Column: Chiralpak – IA (250mX 4.6mm,5 $\mu$ m), RT: 4.56 min, Mobile Phase :0.1% TFA/MeOH, Flow :0.700ml/min.

## EXAMPLE 2

**[0284] Synthesis of (R)-N-hydroxy-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl) phenyl) sulfonamido) pentanamide (41)**



**[0285] Synthesis of methyl N-((4-(methylsulfonylamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (2):** To a stirred solution of methyl N-((4-aminophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (500 mg, 1.27 mmol, 1 eq; for synthesis, see Example 49, below) in pyridine (5 mL) at 0 °C, was added mesyl chloride (160 mg, 1.404 mmol, 1.1 eq), allowed to RT, and then stirred at RT for 1h. The reaction was monitored by TLC; after completion of the reaction, water was added and the RM extracted with DCM (2x100 mL). The combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product purified by column chromatography over silica gel using 3% MeOH/DCM as eluent to obtain an methyl N-((4-(methylsulfonylamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (300.0 mg, 49.7%) as color less liquid. TLC: 5% MeOH/DCM (*R<sub>f</sub>*: 0.6); LCMS: 95.11%, *m/z*=471.0 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 10.42 (s, 1H), 8.52-8.59 (m, 1H), 8.47 (d, *J*=3.55 Hz, 1H), 7.76-7.84 (m, 3H), 7.32-7.40 (m, 3H), 4.64 (d, *J*=16.75 Hz, 1H), 4.38-4.50 (m, 2H), 3.32-3.34 (s, 3H), 3.12 (s, 3H), 1.35-1.51 (m, 2H), 1.22-1.34 (m, 1H), 0.79 (d, *J*=6.48 Hz, 3H), 0.52 (d, *J*=6.60 Hz, 3H).

**[0286] Synthesis of N-((4-(methylsulfonylamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (3):** To a stirred solution of methyl N-((4-(methylsulfonylamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (300 mg, 0.639 mmol, 1 eq) in THF:Water (2:1, 3 mL), LiOH.H<sub>2</sub>O (35 mg, 0.831 mmol, 1.3 eq) was added at RT. The resulting reaction mixture was stirred at RT for 6 h. After consumption of the starting material, volatiles were evaporated, acidified with 5% citric acid solution and extracted

with EtOAc (2x50 mL); the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to obtain N-((4-(methylsulfonamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (270.0 mg, 92.7%), as an off white solid. TLC: 10% MeOH/DCM (*R<sub>f</sub>*: 0.2), LCMS: 98.09%, *m/z*=456.0 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 12.83-12.90 (m, 1H), 10.42 (br s, 1H), 8.59 (br s, 1H), 8.47 (br d, *J*=3.20 Hz, 1H), 7.85 (br d, *J*=7.69 Hz, 1H), 7.77 (d, *J*=8.97 Hz, 2H), 7.35-7.40 (m, 1H), 7.31 (d, *J*=8.33 Hz, 2H), 4.67-4.72 (m, 1H), 4.34-4.45 (m, 2H), 3.12 (s, 3H), 1.38-1.45 (m, 2H), 1.27-1.34 (m, 1H), 0.79 (d, *J*=6.41 Hz, 3H), 0.48 (d, *J*=6.41 Hz, 3H).

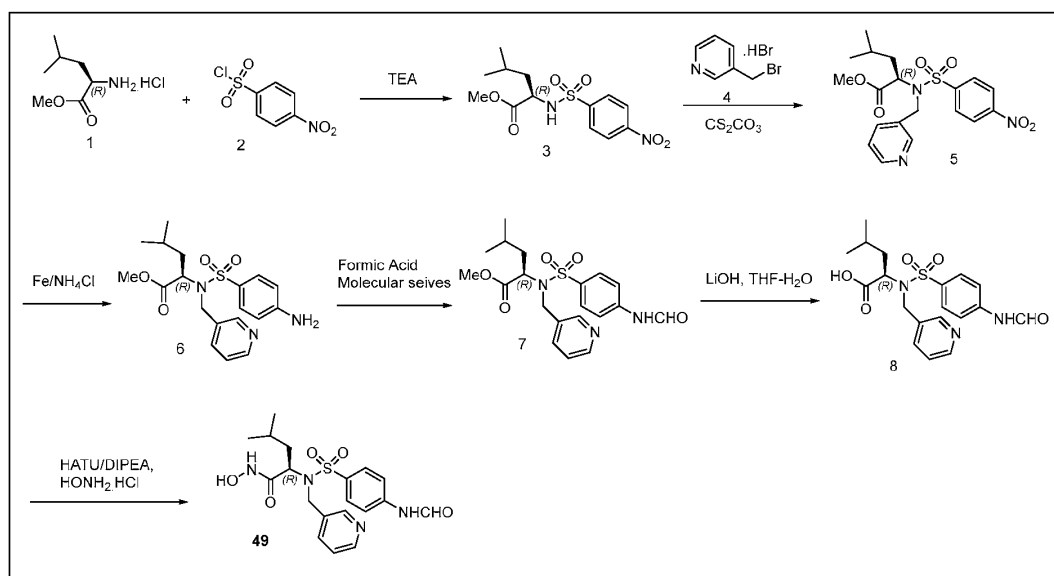
**[0287] Synthesis of (R)-N-(tert-butoxy)-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pentanamide (4):** To a stirred solution of N-((4-(methylsulfonamido)phenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (250 mg, 0.548 mmol, 1 eq) in DCM (10 mL) at 0 °C, were added HATU (271 mg, 0.713 mmol, 1.3 eq), and DIPEA (354 mg, 2.74 mmol, 5 eq); the RM was stirred at 0 °C for 10 min, and N-tert-Butylhydroxylamine hydrochloride (137 mg, 1.09 mmol, 2 eq) added, and warmed to RT and stirred for 14h. After completion of the reaction, the RM was treated with water and extracted with DCM (2x50 mL); the combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to obtain (R)-N-(tert-butoxy)-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl)phenyl)-sulfonamido)pentanamide (70.0 mg, 24.2%) as light brown solid. TLC: 10% MeOH/ DCM (*R<sub>f</sub>*: 0.3); LCMS: 85.7%, *m/z*=527.0 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 10.72 (s, 1H), 10.36-10.41 (m, 1H), 8.51 (s, 1H), 8.41-8.44 (m, 1H), 7.74 (br d, *J*=8.56 Hz, 3H), 7.26-7.31 (m, 3H), 4.67-4.80 (m, 2H), 4.30-4.34 (m, 1H), 3.10 (s, 3H), 1.16-1.18 (m, 2H), 1.13-1.16 (m, 1H), 1.05 (s, 9H), 0.79 (br d, *J*=5.87 Hz, 3H), 0.61 (br d, *J*=5.99 Hz, 3H).

**[0288] Synthesis of (R)-N-hydroxy-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)pentanamide (41):** To a stirred solution of (R)-N-(tert-butoxy)-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl) phenyl)sulfonamido)pentanamide (70 mg, 0.13 mmol, 1 eq) in DCM (5 mL) at 0 °C, was added 1 M BBr<sub>3</sub> in DCM (36 mg, 0.14 mmol, 1.1 eq), warmed to RT and stirred for 3h. The RM was concentrated under reduced pressure to obtain a crude solid which was purified by preparative HPLC to obtain an (R)-N-hydroxy-4-methyl-2-((4-(methylsulfonamido)-N-(pyridin-3-ylmethyl)phenyl)-sulfonamido)pentanamide (41; 10.0 mg, 16.1%) as off-white

solid. TLC: 10% MeOH/ DCM ( $R_f$ : 0.1).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  8.59 (br s, 1H), 8.41-8.46 (m, 1H), 7.94 (br d,  $J=7.82$  Hz, 1H), 7.76 (br d,  $J=8.56$  Hz, 2H), 7.33-7.42 (m, 3H), 4.75-4.82 (m, 2H), 4.36-4.44 (m, 1H), 3.09 (s, 3H), 1.50-1.60 (m, 1H), 1.33-1.42 (m, 2H), 0.88 (br d,  $J=5.99$  Hz, 3H), 0.71 (br d,  $J=6.24$  Hz, 3H); LCMS: 99.38%,  $m/z=470.9$   $[\text{M}+\text{H}]^+$ ; (Column; EVO-C18 (3.0X50mm, 2.6 $\mu\text{m}$ ); RT:1.94 min, A: 2.5 mM Ammonium acetate in water, B: ACN T/B%: 0.01/5, 3/90, 5/90, 5.5/5, 6/5, 0.8 mL/min); HPLC: 99.92%; (Column; X-SELECT CSH C-18; 4.6 x 150 mm, 3.5  $\mu\text{m}$ ); RT:4.66 min, Diluent: ACN:H<sub>2</sub>O.

### EXAMPLE 3

#### [0289] Synthesis of (R)-2-((4-formamido-N-(pyridin-3-ylmethyl)phenyl)-sulfonamido)-N-hydroxy-4-methyl pentanamide (49)



**[0290] Synthesis of methyl ((4-nitrophenyl)sulfonyl)-D-leucinate (3):** To a stirred solution of methyl D-leucinate hydrochloride (1.0 g, 5.50 mmol, 1 eq) in DCM (30 mL) at 0 °C, were added triethylamine (2.78 g, 27.49 mmol, 5 eq), followed by 4-nitrobenzenesulfonyl chloride (1.28 g, 5.78 mmol, 1.05 eq), under  $\text{N}_2$  atmosphere. The RM was stirred at RT for 4 h. Water was added and extracted with DCM (2x100 mL), and

the organic phases are dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using 20% EtOAc/heptane as eluent to obtain an methyl ((4-nitrophenyl)sulfonyl)-D-leucinate (900.0 mg, 49.7%) as brown solid. TLC: 40% EtOAc/heptane (*R<sub>f</sub>*: 0.5); LCMS: 80.41%, *m/z*=331.1 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 8.74 (br d, *J*=7.09 Hz, 1H), 8.41 (d, *J*=8.80 Hz, 2H), 8.00 (d, *J*=8.80 Hz, 2H), 3.81-3.90 (m, 1H), 3.37 (s, 3H), 1.49-1.61 (m, 1H), 1.38-1.49 (m, 2H), 0.83 (d, *J*=6.48 Hz, 3H), 0.70-0.79 (m, 3H).

**[0291] Synthesis of methyl N-((4-nitrophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (5):** To a stirred solution of methyl ((4-nitrophenyl)sulfonyl)-D-leucinate (500 mg, 1.513 mmol, 1 eq) in DMF (5 mL) at RT, were added CS<sub>2</sub>CO<sub>3</sub> (1.48 g, 4.54 mmol, 3 eq), followed by 3-(bromomethyl)pyridine·HBr (440 mg, 1.73 mmol, 1.15 eq), and the RM stirred at RT for 16 h. Water was added and extracted with EtOAc (2x50 mL); the combined organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography over silica gel using 30% EtOAc/heptane as eluent to obtain an methyl N-((4-nitrophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (445.0 mg, 69.8%) as a brown solid. TLC: 50% EtOAc/heptane (*R<sub>f</sub>*: 0.3); LCMS: 93.8%, *m/z*=422.2 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.49-8.60 (m, 2H), 8.32 (d, *J*=8.93 Hz, 2H), 7.91-7.96 (m, 2H), 7.86-7.90 (m, 1H), 7.27-7.29 (m, 1H), 4.65-4.77 (m, 2H), 4.41 (d, *J*=16.38 Hz, 1H), 3.50 (s, 3H), 1.39-1.55 (m, 3H), 0.89-0.93 (m, 3H), 0.59 (d, *J*=6.48 Hz, 3H).

**[0292] Synthesis of methyl N-((4-aminophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (6):** To a stirred solution of methyl N-((4-nitrophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (2 g, 4.74 mmol, 1 eq) in MeOH:water (1:1, 20 mL; two batches of **5**), were added Fe powder (794 mg, 1.42 mmol, 3 eq), NH<sub>4</sub>Cl (760 mg, 1.42 mmol, 3 eq), at RT. The resultant RM was heated to 70 °C for 2 h. The RM was filtered through a celite bed, and washed with EtOAc (2 x 50 mL). Volatiles were evaporated and sat. NH<sub>4</sub>Cl (20 mL) added, and extracted with EtOAc (3 x 50 mL); the combined organic extracts were washed with brine (50 mL); dried over sodium sulfate, filtered and concentrated *in vacuo* to obtain methyl N-((4-aminophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (1.48 g, 80%) as gummy liquid. TLC: 5% MeOH/ DCM (*R<sub>f</sub>*: 0.5). LCMS: 95.4%, *m/z*=392.2 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 8.50-8.57 (m, 1H), 8.46 (dd, *J*=1.22, 4.65 Hz, 1H), 7.79

(br d, J=8.07 Hz, 1H), 7.43 (d, J=8.68 Hz, 2H), 7.36 (dd, J=4.65, 7.58 Hz, 1H), 6.58-6.65 (m, 2H), 6.07 (s, 2H), 4.41-4.57 (m, 2H), 4.37 (dd, J=5.75, 8.93 Hz, 1H), 3.36 (s, 3H), 1.20-1.49 (m, 3H), 0.76 (d, J=6.48 Hz, 3H), 0.54 (d, J=6.60 Hz, 3H).

**[0293] Synthesis of methyl N-((4-formamidophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (7):** To a stirred solution of methyl N-((4-aminophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (100 mg, 0.255 mmol, 1 eq) in formic acid (1 mL), at RT, was added molecular sieves (100 mg), and the RM stirred at 60 °C for 6h. The reaction cooled to RT, water added and extracted with DCM (2x50 mL); the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to obtain methyl N-((4-formamidophenyl)-sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (100.0 mg, 94%) as light brown solid. TLC: 10% MeOH/ DCM (*R<sub>f</sub>*: 0.6); LCMS: 98.2%, *m/z*=420.2 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 8.51-8.58 (m, 2H), 8.45 (d, J=1.22 Hz, 1H), 7.87-8.02 (m, 1H), 7.70-7.79 (m, 4H), 7.37 (br d, J=8.07 Hz, 1H), 7.16 (d, J=8.68 Hz, 1H), 4.61-4.73 (m, 2H), 4.44-4.54 (m, 1H), 3.46-3.49 (m, 3H), 1.49-1.58 (m, 1H), 1.38-1.47 (m, 2H), 0.87-0.90 (m, 3H), 0.58-0.64 (m, 3H).

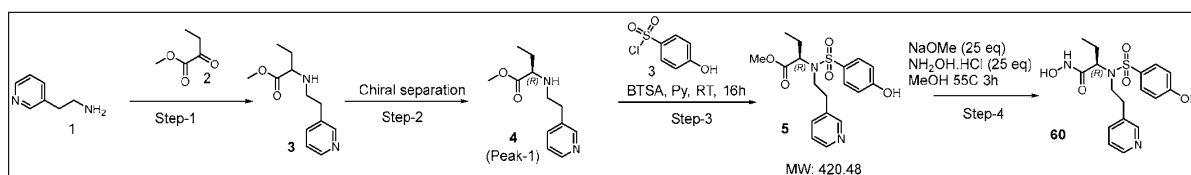
**[0294] Synthesis of N-((4-formamidophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (8):** To a stirred solution of methyl N-((4-formamidophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucinate (600 mg, 1.43 mmol, 1 eq; several combined batches of **7**) in THF:water (2:1, 1.8 mL) was added LiOH.H<sub>2</sub>O (78 mg, 1.85 mmol, 1.3 eq) at RT and the resulting RM was stirred for 6 h. Volatiles were evaporated, acidified with 5% citric acid solution and extracted with EtOAc (2x50 mL); the organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to obtain N-((4-formamidophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (300.0 mg, 51.7%), as an off white solid. TLC: 10% MeOH/DCM (*R<sub>f</sub>*: 0.2), LCMS: 99.4%, *m/z*=406.0 [M+H]<sup>+</sup>; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 12.63-12.88 (m, 1H), 10.48-10.61 (m, 1H), 8.59 (s, 1H), 8.47 (d, J=4.48 Hz, 1H), 8.36 (s, 1H), 7.85 (br d, J=8.33 Hz, 1H), 7.74-7.78 (m, 3H), 7.37 (dd, J=5.12, 7.69 Hz, 2H), 4.68-4.73 (m, 1H), 4.34-4.46 (m, 2H), 1.37-1.46 (m, 2H), 1.27-1.35 (m, 1H), 0.80 (d, J=6.41 Hz, 3H), 0.49 (d, J=6.41 Hz, 3H).

**[0295] Synthesis of (R)-2-((4-formamido-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)-N-hydroxy-4-methylpentanamide (49):** A stirred solution of N-((4-formamidophenyl)sulfonyl)-N-(pyridin-3-ylmethyl)-D-leucine (500 mg,

1.23 mmol, 1 eq; combined batches of **8**) in DMF (3 mL) was cooled to 0 °C, and HATU (703 mg, 1.849 mmol, 1.5 eq) and DIPEA (238 mg, 1.849 mmol, 1.5 eq) added. The RM was stirred at 0 °C for 15 min and NH<sub>2</sub>OH.HCl (85 mg, 1.23 mmol, 1 eq) added. This RM was allowed to warm to RT and was stirred for 6h. The reaction was monitored by TLC (30 % conversion). HATU (1.406 g, 3.699 mmol, 3.0 eq), DIPEA (952 mg, 7.38 mmol, 6.0 eq), and NH<sub>2</sub>OH.HCl (425 mg, 6.15 mmol, 5.0 eq) were added and stirred at RT for 16 h. The RM was added water (5 mL) and extracted with 10 % MeOH in DCM (3x 50 mL). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to give 300 mg of crude solid which was purified by preparative HPLC to obtain an (R)-2-((4-formamido-N-(pyridin-3-ylmethyl)phenyl)sulfonamido)-N-hydroxy-4-methylpentanamide (**49**; 12.0 mg) as colorless syrup. TLC: 10% MeOH/ DCM (*R<sub>f</sub>*: 0.2). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz): δ 10.79-11.00 (br s, 1H), 10.45-10.64 (m, 1H), 8.89-9.01 (m, 1H), 8.48-8.56 (m, 1H), 8.39-8.47 (m, 1H), 8.32-8.39 (m, 1H), 7.61-7.81 (m, 4H), 7.26-7.45 (m, 2H), 4.47-4.80 (m, 2H), 4.15-4.30 (m, 1H), 1.12-1.47 (m, 3H), 0.73-0.81 (m, 3H), 0.56-0.64 (m, 3H); LCMS: 99.61%, *m/z*=421.1 [M+H]<sup>+</sup>; (Column; EVO-C18 (3.0X50mm, 2.6µm); RT:2.51 min, A: 2.5 mM Ammonium acetate, B: ACN T/B%: 0.01/5, 3/95, 5/95, 5.7/5, 0.8 mL/min); HPLC: 98.81%; (Column; X-SELECT CSH C-18 (4.6 x 150 mm, 3.5 µm); RT:4.61 min, Diluent: ACN:H<sub>2</sub>O).

#### EXAMPLE 4

##### Synthesis of (R)-N-hydroxy-2-((4-hydroxy-N-(2-(pyridin-3-yl)ethyl)phenyl)sulfonamido)butanamide (**60**)



**[0296] Step 1: Synthesis of methyl 2-((2-(pyridin-3-yl)ethyl)amino)butanoate:** To a stirred solution of 3-Pyridineethyl amine (**1**) (4 g, 32.70 mmol, 1 eq), in 1,2 -DCE (100 mL), were added methyl Oxo butaroate (**2**) (4.2 g, 32.70 mmol, 1.0 eq), and STAB (10.4 g, 65.40 mmol, 1.5 eq) at 0 °C, then stirred at RT for 14 h. The reaction was monitored

by TLC, after completion of the starting material, the volatiles were concentrated under reduced pressure and the crude was diluted with EtOAc (120 mL), washed with water (3 x 100 mL), brine solution (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain methyl 2-((2-(pyridin-3-yl)ethyl)amino)butanoate (3) (8.1 g, crude racemic compound). Enantiomers were separated by chiral HPLC purification which afforded Peak-1 (desired isomer) (4) 3.0 g as a colourless liquid and Peak-2 undesired isomer 2.5 g as a colourless liquid.

**[0297] Racemic HPLC Condition:** Peak-1 (5.831 min) and Peak-2 (11.196 min) (**Chiral** Pak-ADH (4.6 x 250 mm, 5 µm); {Mobile phase A: 0.1% TFA in n-Hexane B: EtOH (85:15). Flow rate: 1.0 mL/min}).

**[0298] Peak-1: <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)** δ = 8.49 - 8.37 (m, 2H), 7.65 (br d, *J* = 7.8 Hz, 1H), 7.31 (dd, *J* = 7.6, 4.8 Hz, 1H), 3.64 (s, 3H), 3.17 (br t, *J* = 6.4 Hz, 1H), 2.79 - 2.60 (m, 4H), 2.16 - 1.86 (m, 1H), 1.55 (qd, *J* = 13.6, 6.9 Hz, 2H), 0.90 - 0.77 (m, 3H).

**[0299] LCMS:** 96%, *m/z*: [M+H]<sup>+</sup>; mass spec calculated for C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>, 222.29; mass spec found, 223.1 (Column; EVO C-18 (3 x 50 mm, 2.6 µm); RT: 0.194 min; A: 0.5 mL HCOOH in 950 mL H<sub>2</sub>O + 50 mL ACN B: 0.5 mL HCOOH in ACN; T/B%:0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow rate: 1.2 mL/min (Gradient).

**[0300] Optical Rotation:** [α]<sub>D</sub><sup>25</sup> 18.64 (c 0.25, MeOH)

**[0301] Peak-2: <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)** δ = 8.49 - 8.37 (m, 2H), 7.70 - 7.61 (m, 1H), 7.31 (dd, *J* = 7.7, 4.8 Hz, 1H), 3.65 (s, 3H), 3.26 (br s, 1H), 2.85 - 2.67 (m, 4H), 1.66 - 1.50 (m, 2H), 0.84 (t, *J* = 7.4 Hz, 3H).

**[0302] LCMS:** 96%, *m/z*: [M+H]<sup>+</sup>; mass spec calculated for C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>2</sub>, 222.29; mass spec found, 223.1 (Column; EVO C-18 (3 x 50 mm, 2.6 µm); RT: 0.188 min; A: 0.5 mL HCOOH in 950 mL H<sub>2</sub>O + 50 mL ACN B: 0.5 mL HCOOH in ACN; T/B%:0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow rate: 1.2 mL/min (Gradient).

**[0303] Optical Rotation:** [α]<sub>D</sub><sup>25</sup> -20.59 (c 0.25, MeOH).

**[0304] Step 3: Synthesis of methyl (R)-2-((4-hydroxy-N-(2-(pyridin-3-yl)ethyl)phenyl)sulfonamido)butanoate:** To a stirred solution of **Int-4** (4.0 g, 18.04 mmol, 1 eq) in Pyridine (30 mL) was added pre complex solution of 4-hydroxybenzene sulphonylchloride (8.64 g, 45.0 mmol, 2.5 eq) and BTSA (9.1 g, 45.0 mmol) in THF (11 mL) at 0 °C, then stirred at RT for 2.5 h. The reaction was monitored by TLC, after

completion of the reaction, reaction mixture was quenched with ice water (20 ml) and most of the pyridine was evaporated under reduced pressure; reaction mixture was diluted with water (30 ml) and extracted with EtOAc (3 x 50 mL), and combined organic phases were washed with brine (40 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. The crude was purified by column chromatography using Neutral alumina to give (1.1 g, 36%) base on SM recovered.

**[0305] <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)** δ = 10.46 (s, 1H), 8.46 - 8.40 (m, 2H), 7.67 - 7.62 (m, 3H), 7.32 (dd, *J* = 7.7, 4.9 Hz, 1H), 6.92 - 6.88 (m, 2H), 4.25 (dd, *J* = 9.2, 6.0 Hz, 1H), 3.42 (s, 3H), 3.32-3.30 (m, 1H), 2.97 - 2.79 (m, 2H), 2.55-2.45 (m, 1H), 1.88 - 1.77 (m, 1H), 1.64 - 1.52 (m, 1H), 0.80 (t, *J* = 7.3 Hz, 3H)

**[0306] LCMS:** 89.9%, *m/z*: [M+H]<sup>+</sup>; mass spec calculated for C<sub>18</sub>H<sub>22</sub>N<sub>2</sub>O<sub>5</sub>S, 378.44; mass spec found, 379 (Column; X-bridge BEH C-18 (3 x 50 mm, 2.5 μm); RT: 1.35 min; A: 0.5 mL HCOOH in 950 mL H<sub>2</sub>O + 50 mL ACN B: 0.5 mL HCOOH in ACN; T/B%:0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow rate: 0.5 mL/min (Gradient).

**[0307] Step 4: Synthesis (R)-N-hydroxy-2-((4-hydroxy-N-(2-(pyridin-3-yl)ethyl)phenyl)sulfonamido)butanamide (60):** To a stirred solution of methyl (R)-2-((4-hydroxy-N-(2-(pyridin-3-yl)ethyl)phenyl)sulfonamido)butanoate (5) (1.1 g, 2.90 mmol, 1.0 eq) in MeOH (15 mL) was added 17.6 mL of NaOMe (72.5 mmol, 25.0 eq, 3.2M in MeOH) and NH<sub>2</sub>OH.HCl (5.0 g, 72.5 mmol 25 eq) at RT, then stirred at 55°C for 3 h. The reaction was monitored by TLC, after completion of the reaction the salts were removed by filtration and the methanol was concentrated under reduced pressure. The product was purified by prep HPLC followed by lyophilisation to afford 400 mg of (R)-N-hydroxy-2-((4-hydroxy-N-(2-(pyridin-3-yl)ethyl)phenyl)sulfonamido) butanamide) (**60**; 68%) as an off white solid.

**[0308] <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)** δ = 10.08 (s, 2H), 8.98 - 8.89 (m, 1H), 8.45 - 8.40 (m, 2H), 7.71 - 7.59 (m, 3H), 7.36 - 7.27 (m, 1H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.06 - 3.94 (m, 1H), 3.69 - 3.56 (m, 1H), 3.26 - 3.17 (m, 1H), 2.96 - 2.80 (m, 2H), 1.73 - 1.59 (m, 1H), 1.40 - 1.31 (m, 1H), 0.70 (t, *J* = 7.3 Hz, 3H)

**[0309] LCMS:** 99.74%, *m/z*: [M+H]<sup>+</sup>; mass spec calculated for C<sub>17</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>S, 379.43; mass spec found, 380 (Column; X-bridge BEH C-18 (3 x 50 mm, 2.5 μm); RT: 0.98 min;

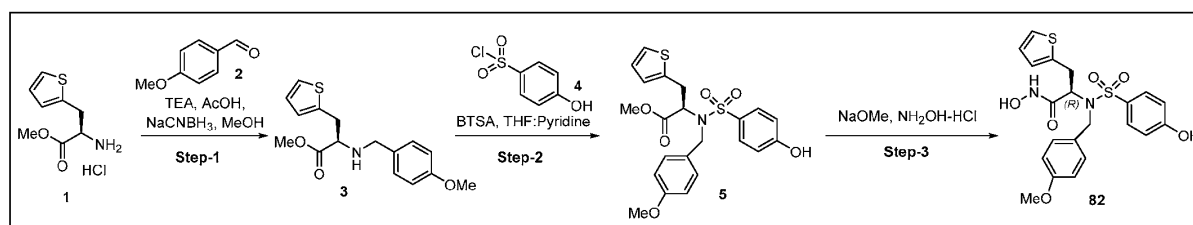
A: 0.5 mL HCOOH in 950 mL H<sub>2</sub>O + 50 mL ACN B: 0.5 mL HCOOH in ACN; T/B%:0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow rate: 1.2 mL/min (Gradient).

**[0310] HPLC:** 99.93% (Column: X-Select CSH C-18 (4.6 x 150 mm, 3.5  $\mu$ m); {Mobile phase A: 0.1% HCOOH in water: ACN (95:05): B: ACN Flow rate: 1.2 mL/min); Gradient Programme: T/B%: 0.01/2, 2/2, 12/90, 16/90).

**[0311] Optical rotation:**  $[\alpha]_D^{25}$  11.94 (*c* 0.06, MeOH).

## EXAMPLE 5

### **[0312] Synthesis of (R)-N-hydroxy-2-((4-hydroxy-N-(4-methoxybenzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanamide (82)**



### **[0313] Synthesis of methyl (R)-2-((4-methoxybenzyl) amino)-3-(thiophen-2-yl) propanoate (3):**

To a stirred solution of methyl (R)-2-amino-3-(thiophen-2-yl) propanoate hydrochloride (1) (1.2 g, 5.42 mmol, 1 eq), in MeOH (15 mL), were added TEA (0.55 g, 5.42 mmol, 1 eq), 4-methoxybenzaldehyde (2) (0.88 g, 6.51 mmol, 1.2 eq), and acetic acid (1.62 mL, 27.1 mmol 5 eq), then allowed to stirred at 60°C for 16h. Further, reaction mixture cooled to 0°C and added NaCNBH<sub>3</sub> (0.85 g, 13.55 mmol, 2.5 eq), was portion wise, reaction mixture stirred for 3h at RT. The reaction was monitored by TLC, after completion of the reaction, water was added and extracted with DCM (2 x 100 mL), the organic phases were washed with saturated solution of NaHCO<sub>3</sub> (30 mL), and brine solution (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain crude, The crude product was purified by combi flash using 40% EtOAc/Heptane as an eluent to obtain methyl (R)-2-((4-methoxybenzyl) amino)-3-(thiophen-2-yl) propanoate (3) (1.25 g, 75%), as color less oil.

**[0314] <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):** δ 7.33 - 7.31 (m, 1H), 7.22 - 7.18 (m, 2H), 6.94 - 6.90 (m, 1H), 6.87 - 6.83 (m, 3H), 3.72 (s, 3H), 3.71 - 3.65 (m, 1H), 3.60 (s, 3H), 3.58 - 3.55 (m, 1H), 3.44 - 3.35 (m, 1H), 3.16 - 3.04 (m, 2H), 2.45 - 2.39 (m, 1H). **LCMS:** 93.4%, m/z: [M+H]<sup>+</sup>; mass spec calculated for C<sub>16</sub>H<sub>19</sub>NO<sub>3</sub>S, 305.11; mass spec found, 306.1

**[0315] Synthesis of methyl (R)-2-((4-hydroxy-N-(4-methoxybenzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanoate (5):** To a stirred solution of 4-hydroxybenzenesulfonyl chloride (4) (1.96 g, 10.22 mmol, 2.5 eq), in THF (10 mL), was added BTSA (2.07 g, 10.22 mmol, 2.5 eq), at 0°C. The reaction mixture was stirred for 2.5h at RT. Further, this reaction mixture was added to the stirred solution of methyl (R)-2-((4-methoxybenzyl) amino)-3-(thiophen-2-yl) propanoate (3) (1.25 g, 4.09 mmol, 1 eq), in pyridine (10 mL), at 0°C, the reaction mixture was stirred overnight at RT. The reaction was monitored by TLC, after completion of the reaction, water was added and extracted with EtOAc (2 x 150 mL), the organic phases are washed with brine solution (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain crude, The crude product was purified by combi flash using 60% EtOAc/heptane as eluent to obtain methyl (R)-2-((4-hydroxy-N-(4-methoxybenzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanoate (5) (280 mg, 15 %), as light pale yellow solid.

**[0316] LCMS:** 74.71%, m/z: [M-H]<sup>-</sup>; mass spec calculated for C<sub>22</sub>H<sub>23</sub>NO<sub>6</sub>S<sub>2</sub>, 461.10; mass spec found, 459.9

**[0317] Synthesis of (R)-N-hydroxy-2-((4-hydroxy-N-(4-methoxy benzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanamide (82):** To a stirred solution of methyl (R)-2-((4-hydroxy-N-(4-methoxybenzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanoate (5) (270 mg, 0.58 mmol, 1 eq), in MeOH (2 mL), were added NH<sub>2</sub>OH-HCl (610 mg, 8.78 mmol, 15 eq), and NaOMe (474 g, 8.78 mmol, 15 eq), then allowed to stir at 0°C for 5 min, then stirred at RT for overnight, The reaction was monitored by TLC, after completion of the reaction, extracted with ethyl acetate and concentrated. The crude product was purified by prep HPLC to obtain (R)-N-hydroxy-2-((4-hydroxy-N-(4-methoxybenzyl) phenyl) sulfonamido)-3-(thiophen-2-yl) propanamide (82) (140 mg, 51%), as an off white solid.

**[0318] <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):** δ 10.94 - 10.38 (m, 2H), 8.96 - 8.88 (m, 1H), 7.60 - 7.56 (m, 2H), 7.33 - 7.30 (m, 1H), 7.23 - 7.18 (m, 2H), 6.91 - 6.87 (m, 1H), 6.85 - 6.78

(m, 4H), 6.70 - 6.68 (m, 1H), 4.51 (s, 2H), 4.45 - 4.40 (m, 1H), 3.72 (s, 3H), 3.25 - 3.17 (m, 1H), 2.62 - 2.59 (m, 1H).

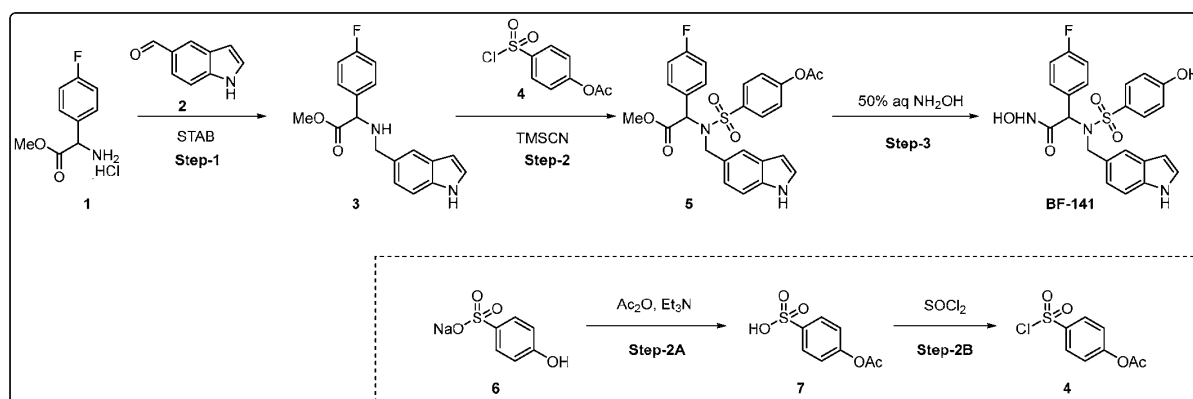
**[0319] LCMS:** 98.54%, m/z: [M-H]<sup>-</sup>; mass spec calculated for C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>6</sub>S<sub>2</sub>, 462.09; mass spec found, 461.1. (Column; X-Select CSH (3.0X50mm, 2.5μm); RT: 1.77 min; A: 0.025% Formic acid in water, B: ACN; Gradient program:

**[0320]** 0% B to 98 % B in 2.0 min, hold till 3.0 min, at 3.2 min B con is 0% up to 4.0 min; Inj Volume: 2.0μL; Flow: 1.2 mL/min; Column oven temperature: 50°C.

**[0321] HPLC:** 99.54%; (Column; X-SELECT CSH C-18 (4.6 x 150 mm, 3.5 μm); RT: 7.03 min; A: 0.1% Formic acid in Water: ACN (95:05), B: ACN; T/B%: 0.01/5, 1/5, 8/100, 12/100, 14/5, 18/5; Flow: 1.2 mL/min.

## EXAMPLE 6

### **[0322] Synthesis of 2-((N-((1H-indol-5-yl) methyl)-4-hydroxy phenyl) sulfonamido)-2-(4-fluorophenyl)-N-hydroxy acetamide (88; BF-141)**



**[0323] Step 1: Synthesis of methyl 2-(((1H-indol-5-yl)methyl)amino)-2-(4-fluorophenyl)acetate (3):** To a stirred solution of methyl 2-amino-2-(4-fluorophenyl)acetate hydrochloride (1) (2.0 g, 9.13 mmol, 1 eq), in 1, 2-DCE (30 mL) was added TEA (1.27 mL, 9.13 mmol), Indole-5-carboxaldehyde (2) (1.59 g, 10.95 mmol, 1.2 eq), and STAB (2.90 g, 13.69 mmol, 1.5 eq) at 0°C, then the reaction mixture was stirred at RT for 16h. The reaction was monitored by TLC, after completion of the reaction, water was added and extracted with DCM (2 x 100 mL), the combined organic phases were

washed with saturated  $\text{NaHCO}_3$  solution (30 mL), and brine solution (20 mL), dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated to obtain crude. The crude product was purified by combi flash using 50% EtOAc/heptane as eluent to obtain methyl 2-(((1H-indol-5-yl)methyl)amino)-2-(4-fluorophenyl)acetate (3) (1.4 g, 49%), as color less oil.

**[0324]  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz):**  $\delta$  10.98 (brs, 1H), 7.46-7.40 (m, 3H), 7.32-7.27 (m, 2H), 7.22-7.13 (m, 2H), 7.04 - 6.98 (m, 1H), 6.37-6.34 (m, 1H), 4.39-4.35 (m, 1H), 3.68-3.64 (m, 2H), 3.58 (s, 3H), 2.94-2.85 (m, 1H).

**[0325] LCMS:** 97.45%, m/z:  $[\text{M}+\text{H}]^+$ ; mass spec calculated for  $\text{C}_{18}\text{H}_{17}\text{FN}_2\text{O}_2$ , 312.13; mass spec found, 313.0

**[0326] Step 2A: Synthesis of 4-acetoxibenzenesulfonic acid (7):** To a stirred solution of sodium 4-hydroxybenzenesulfonate (6) (24 g, 0.12 mol, 1 eq), in Triethylamine (90 mL), was added Acetic anhydride (28.8 mL, 0.3 mol, 2.5 eq), at  $0^\circ\text{C}$ , then stirred at RT for 16h. The reaction was monitored by TLC, after completion of the reaction, the volatiles were concentrated under reduced pressure, co-distilled with toluene (3x, 100 mL), to obtain 4-acetoxibenzenesulfonic acid (7) (22 g, 85%), as pale brown gummy solid.

**[0327]  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz):**  $\delta$  7.71-7.54 (m, 2H), 7.11-7.02 (m, 2H), 2.26 (s, 3H).

**[0328] Step 2B: Synthesis of 4-(Chloro sulfonyl) phenyl acetate (4):** To a stirred solution of 4-acetoxibenzenesulfonic acid (7) (22 g, 0.10 mol, 1eq), in Thionyl chloride (200 mL), was added DMF (0.3 mL), at  $0^\circ\text{C}$ , then heated to  $60^\circ\text{C}$  for 12h. The reaction was monitored by TLC, after completion of the reaction, the volatiles were concentrated under reduced pressure. Diluted with ethyl acetate (1L), washed with water (2 x 500 mL), and concentrated to obtain the 4-(Chloro sulfonyl) phenyl acetate (4) (20 g, 76%), as brown solid.

**[0329]  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz):**  $\delta$  7.66-7.59 (m, 2H), 7.10-7.04 (m, 2H), 2.26 (s, 3H).

**[0330] Step 2: Synthesis of methyl 2-((N-((1H-indol-5-yl) methyl)-4-acetoxy phenyl) sulfonamido)-2-(4-fluoro phenyl) acetate (5):** To a stirred solution of methyl 2-(((1H-indol-5-yl) methyl) amino)-2-(4-fluoro phenyl) acetate (3) (1.4 g, 4.48 mmol, 1 eq) in ACN (20 mL) was added 4-Acetoxibenzenesulfonyl chloride (4) (1.57 g, 6.72 mmol, 1.5 eq) and Trimethylsilylcyanide (1.33 g, 13.44 mmol, 3.0 eq) at RT, Then reaction mixture was

stirred overnight at RT. The reaction was monitored by TLC, after completion of the reaction, water was added and extracted with EtOAc (2 x 75 mL), the combined organic phases are washed with brine solution (20 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain crude. The crude product was purified by combi flash using 5%EtOAc/DCM as eluent to obtain methyl 2-((N-((1H-indol-5-yl) methyl)-4-acetoxy phenyl) sulfonamido)-2-(4-fluoro phenyl) acetate (5) (1 g, 44%), as light pale yellow solid.

**[0331] LCMS:** 93.43%, m/z: [M+H]<sup>+</sup>; mass spec calculated for C<sub>26</sub>H<sub>23</sub>FN<sub>2</sub>O<sub>6</sub>S, 510.13; mass spec found, 511.9

**[0332] Step 3: Synthesis of 2-((N-((1H-indol-5-yl) methyl)-4-hydroxy phenyl) sulfonamido)-2-(4-fluoro phenyl)-N-hydroxy acetamide (88; BF-141):** To a stirred solution of methyl 2-((N-((1H-indol-5-yl) methyl)-4-acetoxy phenyl) sulfonamido)-2-(4-fluoro phenyl) acetate (5) (250 mg, 0.49 mmol, 1 eq), in DMSO (2 mL), were added 50% aqueous NH<sub>2</sub>OH (1.0 mL), then stirred at 55°C for 12h. The reaction was monitored by TLC, after completion of the reaction, reaction mixture was extracted with EtOAc (30 mL) and concentrated to obtain the crude product. The product was purified by prep HPLC to obtain the 2-((N-((1H-indol-5-yl) methyl)-4-hydroxy phenyl) sulfonamido)-2-(4-fluorophenyl)-N-hydroxy acetamide (88; BF-141) (18 mg, 8%), as off white solid.

**[0333] <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 400 MHz):** δ 11.01-10.21 (m, 3H), 9.11-8.94 (m, 1H), 7.49-7.42 (m, 2H), 7.26-7.19 (m, 3H), 7.09-6.99 (m, 4H), 6.79-6.73 (m, 2H), 6.62-6.59 (m, 1H), 6.22-6.19 (m, 1H), 5.43-5.40 (m, 1H), 4.75-4.55 (m, 2H).

**[0334] LCMS:** 98.67%, m/z: [M-H]<sup>-</sup>; mass spec calculated for C<sub>23</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>5</sub>S, 469.11; mass spec found, 468.2 (Column; X-BRIDGE BEH C-18 (3.0x50mm, 2.5μm); RT: 1.85 min; A: 0.025% Formic acid in water, B: ACN; T/B%: 0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow rate: 1.2 mL/min (Gradient), Column temp: 50°C.

**[0335] HPLC:** 99.96%; (Column; X-SELECT CSH C-18 (4.6 x 150 mm, 3.5 μm); RT: 6.79 min; A: 0.1% Formic acid in Water: ACN (95:05), B: ACN; T/B%: 0.01/5, 1/5, 8/100, 12/100, 14/5, 18/5; Flow: 1.2 mL/min.

## Example 7

**[0336] Screening Compounds for Inhibition of BFT *in vitro* - NFF-3 cleavage assay**

**[0337]** A NFF-3 cleavage assay was used to test activity of recombinant BFT, before or after addition of various inhibitors. The NFF-3 cleavage assay was previously described by Goulas et al., *PNAS*, 2011, 108(5) 1856-1861, which is incorporated by reference herein in its entirety.

**[0338]** Initially, recombinant BFT (rBFT) (0.25, 0.5, 1, 2, 4, 8, or 16 µg/mL) was incubated at 37°C with the fluorogenic substrate NFF-3 (Cayman Chemical) at a concentration of 2.5 µM, 5 µM, or 10 µM. After 18 hours, fluorescence was measured in a microplate fluorimeter. As shown in FIG. 5A, a dose-dependent response was observed.

**[0339]** Next, rBFT was pre-incubated with one or more test compounds at different concentrations for 30 minutes at 37°C. The rBFT-compound mixture was then added to NFF-3 and incubated for 24 hours at 37°C. Fluorescence was then measured in a microplate fluorimeter.

**[0340]** FIG. 5B shows dose-inhibition curve of BFT-induced NFF-3 hydrolysis by 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrohydroxamic acid (referred to as OH-CGS or OH-CGS-27023A; see FIG. 3A). The measured IC<sub>50</sub> value is shown in Table 9.

**Table 9.** Inhibition of BFT-induced NFF-3 release by OH-CGS

| Compound Name                                                                       | Alternative names     | CAS #       | IC <sub>50</sub> (µM) |
|-------------------------------------------------------------------------------------|-----------------------|-------------|-----------------------|
| 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrohydroxamic acid | OH-CGS-27023A; OH-CGS | 779342-04-0 | 0.366                 |

**[0341]** The NFF-3 cleavage assay was also used to test the inhibition activity of compounds of the present disclosure (e.g., compounds of Formula I). The data provided in Table 10 (below) shows that these compounds are potent inhibitors of BFT *in vitro*.

## EXAMPLE 8

**[0342]** Screening Compounds for Inhibition of BFT in cells - E-cadherin release assay

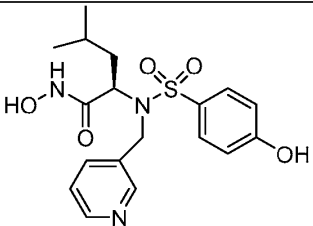
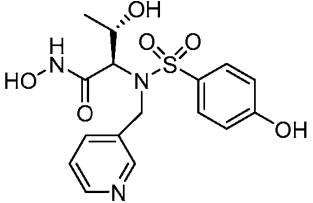
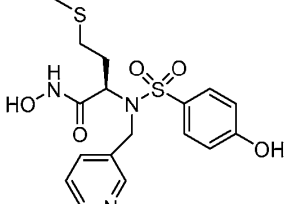
**[0343]** Test compounds were also screened for their ability to inhibit BFT-induced E-cadherin release from HT29 cells.

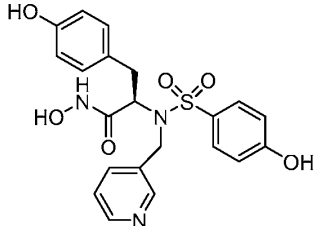
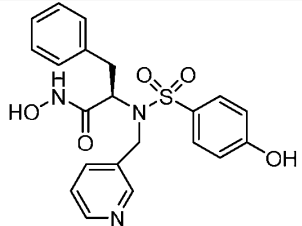
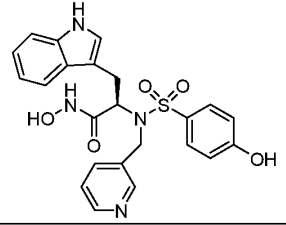
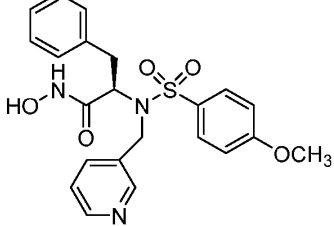
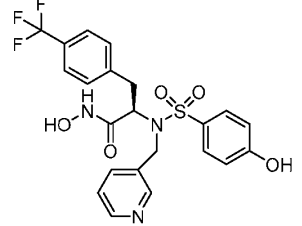
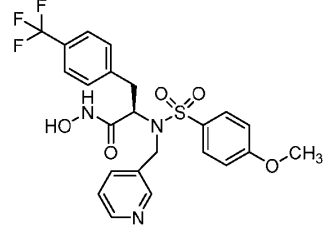
**[0344]** Different concentrations of test compounds were pre-incubated with rBFT for one hour at 37°C. The rBFT-compound mixture was then added to HT29 cells and incubated at 37°C for 18 hours. Following incubation, cell supernatants were harvested and E-cadherin was quantified in the supernatants by ELISA (FIG. 2).

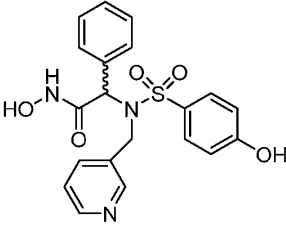
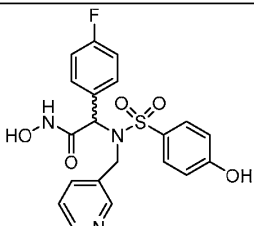
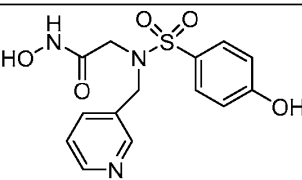
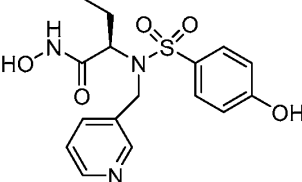
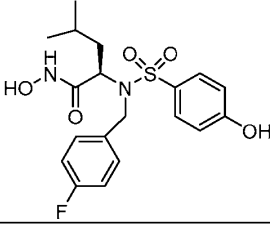
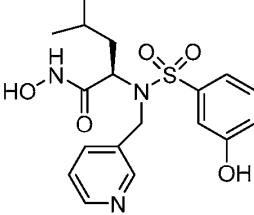
**[0345]** As shown in FIG. 4, OH-CGS-27023A (see FIG. 3A) inhibited BFT with an IC<sub>50</sub> of  $1.99 \times 10^{-6}$  M.

**[0346]** The E-cadherin release assay was also used to test the inhibition activity of compounds of the present disclosure (e.g., compounds of Formula I). The data provided in Table 10 (below) shows that these compounds are potent inhibitors of BFT *in vitro*.

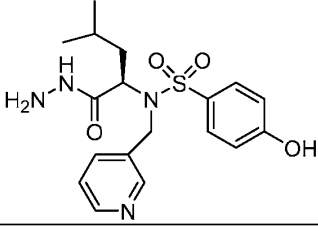
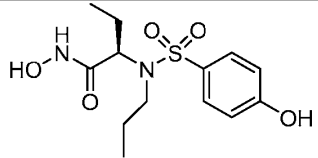
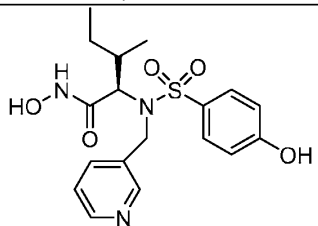
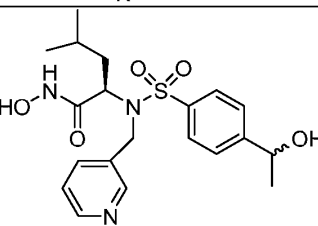
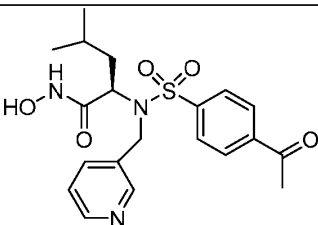
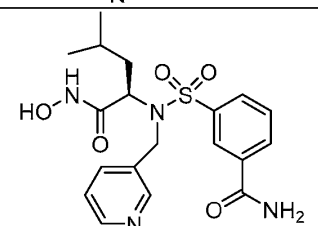
**Table 10.** BFT activity of small molecule inhibitors of the present disclosure.

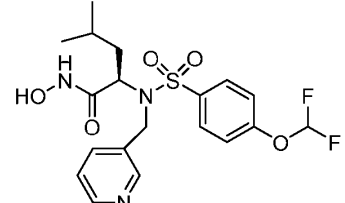
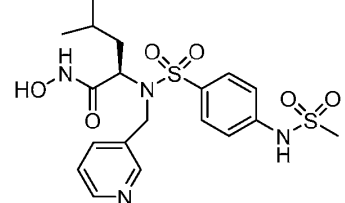
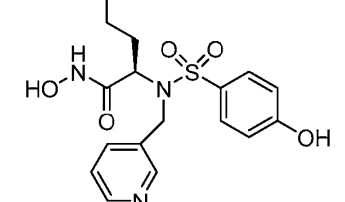
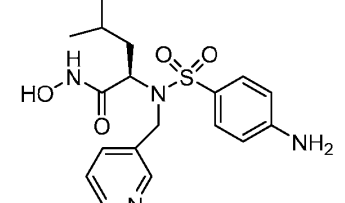
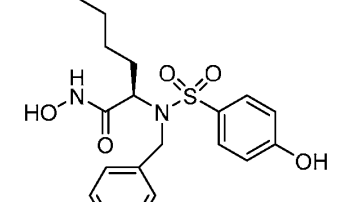
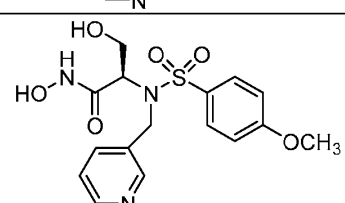
| Cmpd #* | NFF3<br>IC <sub>50</sub><br>( $\mu$ M) | HT29 Cell<br>IC <sub>50</sub> ( $\mu$ M) | HPLC<br>Retention<br>Time (Min) <sup>a</sup> | LC-MS<br>(M+H) | Structure                                                                             |
|---------|----------------------------------------|------------------------------------------|----------------------------------------------|----------------|---------------------------------------------------------------------------------------|
| 1       | <0.15                                  | 1.52                                     | 4.28                                         | 394.20         |  |
| 2       | >1                                     | 6.72                                     | 3.51                                         | 382.20         |  |
| 3       | 0.24                                   | 3.84                                     | 5.48                                         | 412.20         |  |

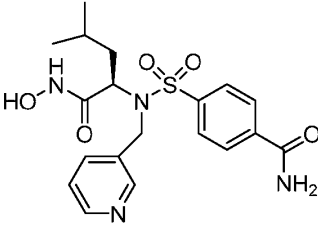
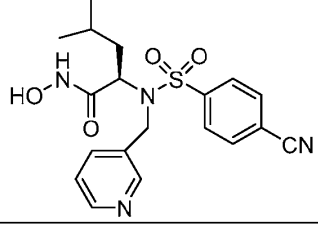
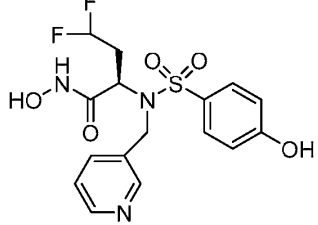
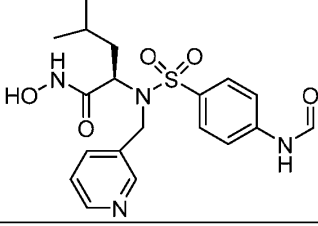
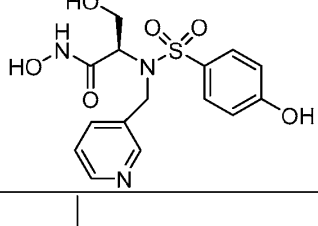
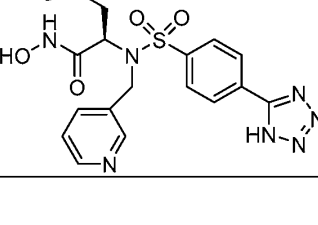
|    |      |      |      |        |                                                                                       |
|----|------|------|------|--------|---------------------------------------------------------------------------------------|
| 4  | 2.04 | 6.33 | 3.82 | 443.90 |     |
| 5  | 0.24 | 4.91 | 4.95 | 428.20 |    |
| 6  | 0.15 | 1.62 | 3.72 | 467.20 |   |
| 19 | 1.24 | 8.26 | 5.72 | 442.10 |   |
| 20 | 0.86 | 7.32 | 5.87 | 496.20 |  |
| 21 | 2.75 | >16  | 7.04 | 510.30 |   |

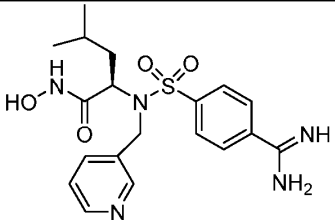
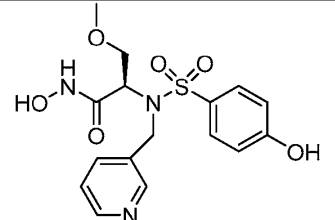
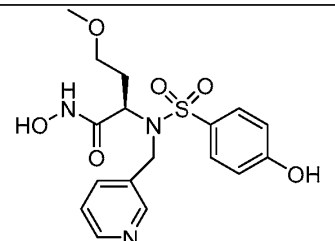
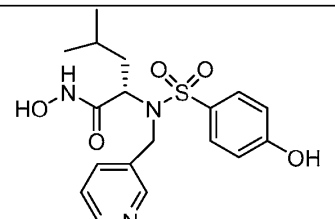
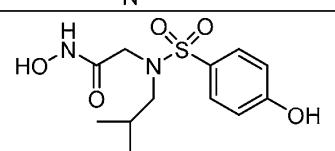
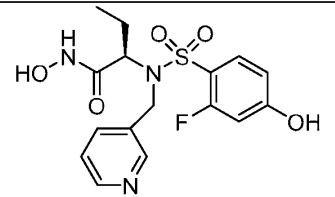
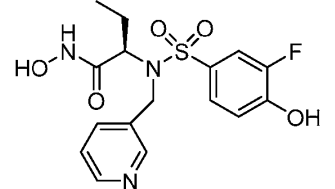
|    |       |       |      |        |                                                                                       |
|----|-------|-------|------|--------|---------------------------------------------------------------------------------------|
| 22 | 0.66  | 17.26 | 4.04 | 414.20 |    |
| 23 | 0.30  | >50   | 6.20 | 432.20 |    |
| 24 | 1.10  | 19.1  | 3.74 | 338.10 |    |
| 25 | 0.090 | 0.78  | 4.48 | 366.20 |   |
| 26 | 6.21  | 1.28  | 8.78 | 409.20 |  |
| 27 | 5.95  | >50   | 4.89 | 394.10 |  |

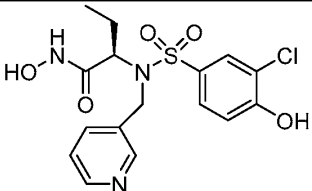
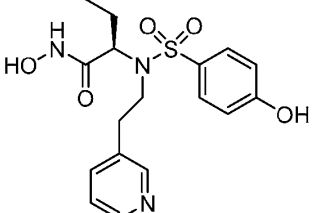
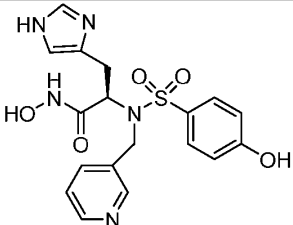
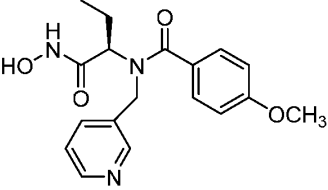
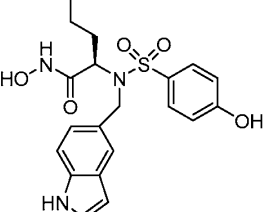
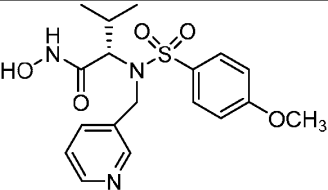
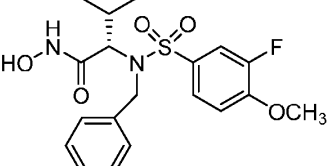
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|----|-------|-------|------|--------|--|
| 28 | 0.14  | 0.198 | 5.33 | 395.00 |  |
| 29 | 0.94  | 3.36  | 4.18 | 401.20 |  |
| 30 | 0.27  | 0.97  | 7.17 | 401.00 |  |
| 31 | 20.06 | >50   | 7.45 | 408.10 |  |
| 32 | 0.51  | 5.17  | 6.39 | 384.42 |  |
| 33 | 0.14  | 1.74  | 5.57 | 432.20 |  |

|    |       |  |      |        |                                                                                      |
|----|-------|--|------|--------|--------------------------------------------------------------------------------------|
| 34 | 28.0  |  | 7.34 | 393.10 |    |
| 35 | 0.94  |  | 5.60 | 316.10 |    |
| 36 | 0.24  |  | 7.90 | 394.00 |    |
| 37 | 49.70 |  | 4.76 | 422.10 |   |
| 38 | 6.13  |  | 4.81 | 420.20 |  |
| 39 | 79.33 |  | 4.00 | 421.20 |  |

|    |       |  |      |        |                                                                                      |
|----|-------|--|------|--------|--------------------------------------------------------------------------------------|
| 40 | 3.63  |  | 5.75 | 442.00 |    |
| 41 | 24.10 |  | 4.66 | 470.90 |    |
| 42 | 0.090 |  | 3.78 | 380.00 |    |
| 43 | 2.99  |  | 4.22 | 393.30 |  |
| 44 | 0.13  |  | 4.83 | 394.30 |  |
| 45 | 0.82  |  | 3.84 | 396.30 |  |

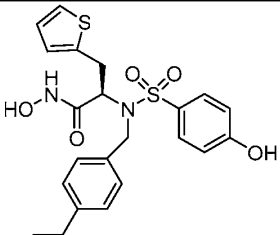
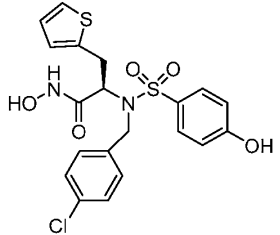
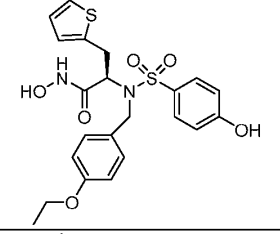
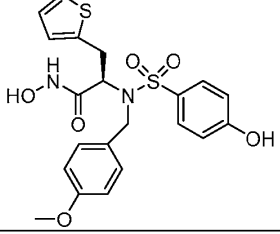
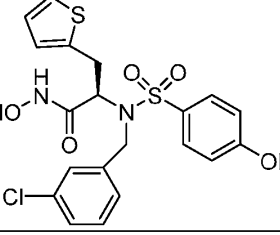
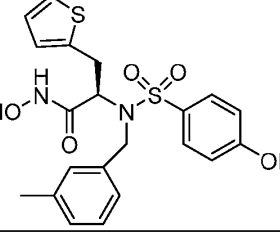
|    |       |  |      |        |                                                                                      |
|----|-------|--|------|--------|--------------------------------------------------------------------------------------|
| 46 | 6.10  |  | 3.81 | 421.20 |    |
| 47 | 6.09  |  | 5.50 | 403.30 |    |
| 48 | 14.60 |  | 3.89 | 402.10 |   |
| 49 | 8.20  |  | 4.61 | 421.10 |  |
| 50 | 0.51  |  | 6.48 | 368.00 |  |
| 51 | >200  |  | 9.28 | 446.10 |  |

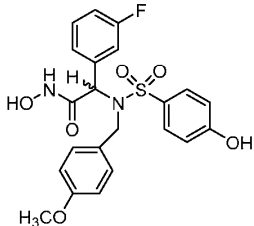
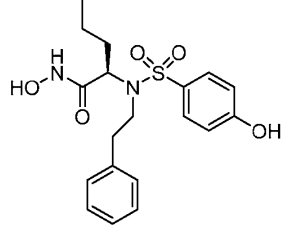
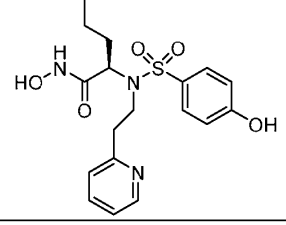
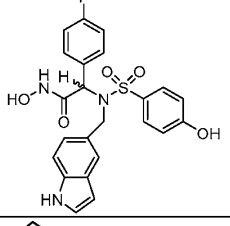
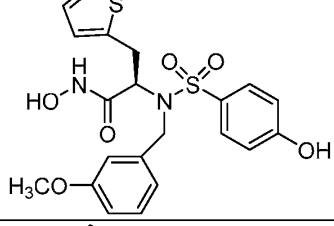
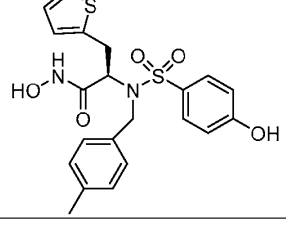
|    |       |  |      |        |                                                                                      |
|----|-------|--|------|--------|--------------------------------------------------------------------------------------|
| 52 | 27.86 |  | 7.67 | 420.10 |    |
| 53 | 0.57  |  | 4.87 | 382.00 |    |
| 54 | 0.40  |  | 2.76 | 396.20 |   |
| 55 | 15.53 |  | 4.88 | 378.00 |  |
| 56 | 4.62  |  | 6.26 | 303.20 |  |
| 57 | 0.196 |  | 7.19 | 384.10 |  |
| 58 | 0.510 |  | 7.16 | 384.10 |  |

|    |        |  |      |        |                                                                                       |
|----|--------|--|------|--------|---------------------------------------------------------------------------------------|
| 59 | 4.22   |  | 7.27 | 400.00 |    |
| 60 | 0.0188 |  | 4.85 | 380.10 |    |
| 61 | 49.23  |  | 9.71 | 417.10 |    |
| 62 | 96.29  |  | 7.31 | 344.10 |   |
| 63 | 0.222  |  | 8.02 | 416.10 |  |
| 64 | 8.28   |  | 5.94 | 394.10 |   |
| 65 | 28.48  |  | 8.83 | 411.00 |   |

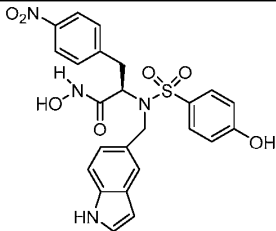
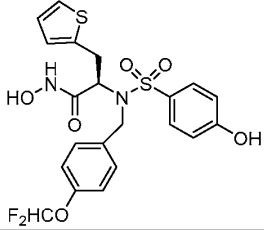
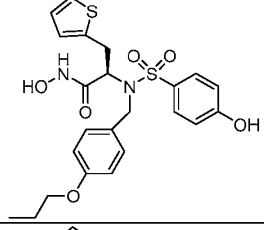
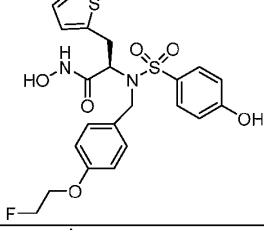
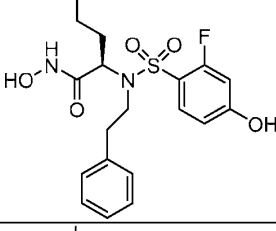
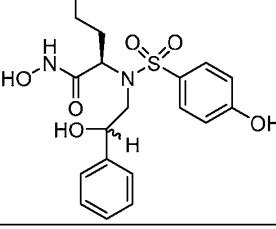
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|----|-------|--|-------|--------|--|
| 66 | 1.34  |  | 4.38  | 382.00 |  |
| 67 | 4.25  |  | 8.40  | 419.00 |  |
| 68 | 50.12 |  | 5.92  | 397.10 |  |
| 69 | 691.3 |  | 11.11 | 430.90 |  |
| 70 | 164.0 |  | 1.57  | 405.20 |  |
| 71 | 3.76  |  | 8.42  | 467.20 |  |
| 72 | 637.2 |  | 11.08 | 452.90 |  |

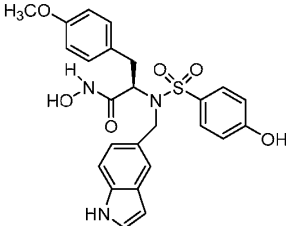
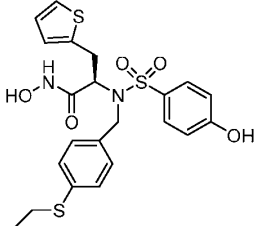
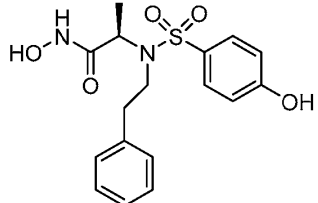
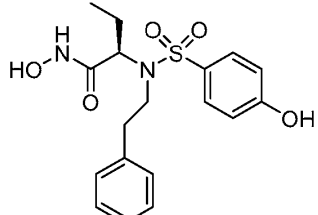
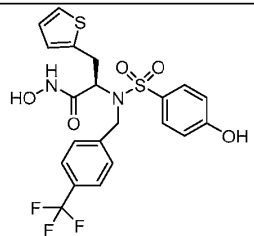
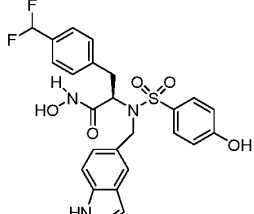
|    |       |  |      |        |  |
|----|-------|--|------|--------|--|
| 73 | NT    |  | 6.04 | 382.20 |  |
| 74 | NT    |  | 9.39 | 396.00 |  |
| 75 | >100  |  | 7.48 | 411.00 |  |
| 76 | 9.93  |  | 5.96 | 370.10 |  |
| 77 | 0.15  |  | 8.38 | 516.90 |  |
| 78 | 0.216 |  | 4.65 | 394.10 |  |

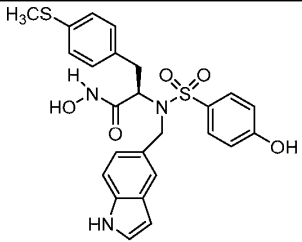
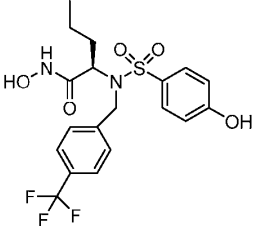
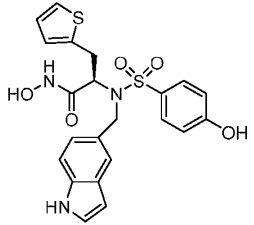
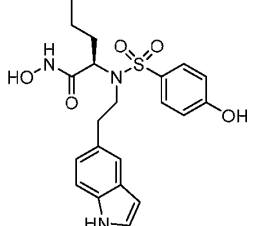
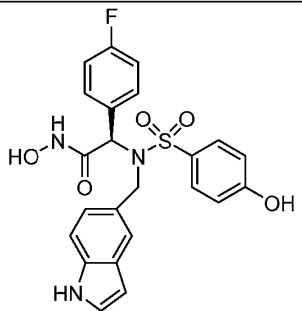
|    |       |  |       |        |                                                                                       |
|----|-------|--|-------|--------|---------------------------------------------------------------------------------------|
| 79 | 0.387 |  | 10.57 | 460.90 |    |
| 80 | 0.290 |  | 7.54  | 466.90 |    |
| 81 | 0.556 |  | 7.37  | 476.90 |   |
| 82 | 0.268 |  | 7.03  | 461.10 |  |
| 83 | 0.530 |  | 7.47  | 466.90 |  |
| 84 | 0.491 |  | 7.36  | 447.00 |  |

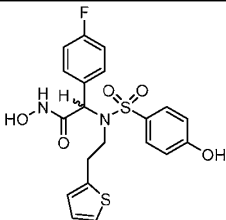
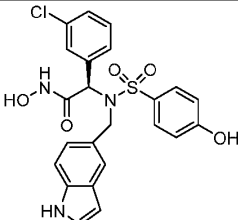
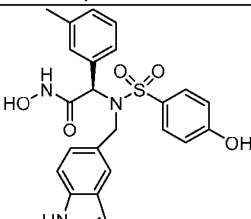
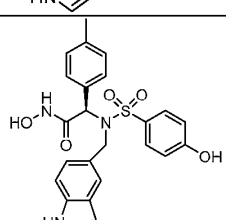
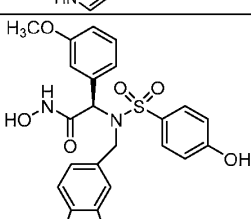
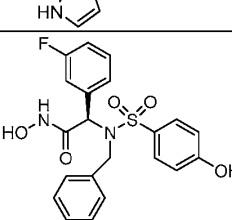
|    |       |  |      |        |                                                                                       |
|----|-------|--|------|--------|---------------------------------------------------------------------------------------|
| 85 |       |  | 7.00 | 459.20 |    |
| 86 |       |  | 7.15 | 393.20 |    |
| 87 |       |  | 4.02 | 394.20 |   |
| 88 | 0.118 |  | 6.79 | 468.20 |  |
| 89 | 0.185 |  | 7.06 | 462.90 |   |
| 90 | 0.236 |  | 8.97 | 447.20 |  |

|    |       |  |      |        |  |
|----|-------|--|------|--------|--|
| 91 | 0.236 |  | 5.16 | 474.20 |  |
| 92 | 0.099 |  | 7.08 | 425.20 |  |
| 93 | 0.117 |  | 6.63 | 482.10 |  |
| 94 | 0.434 |  | 7.42 | 477.25 |  |
| 95 | 0.062 |  | 7.11 | 421.20 |  |
| 96 | 0.088 |  | 7.19 | 421.20 |  |

|     |       |  |      |        |                                                                                       |
|-----|-------|--|------|--------|---------------------------------------------------------------------------------------|
| 97  | 1.123 |  | 6.88 | 509.20 |    |
| 98  | 1.396 |  | 1.93 | 499.05 |    |
| 99  | 0.335 |  | 7.68 | 489.20 |   |
| 100 | 0.150 |  | 7.65 | 495.00 |  |
| 101 | 0.104 |  | 7.50 | 409.21 |  |
| 102 | 0.068 |  | 5.96 | 407.30 |  |

|     |       |  |      |        |                                                                                       |
|-----|-------|--|------|--------|---------------------------------------------------------------------------------------|
| 103 | 0.483 |  | 7.32 | 494.30 |    |
| 104 | 0.810 |  | 7.05 | 491.30 |    |
| 105 | 0.090 |  | 5.89 | 365.20 |    |
| 106 | 0.038 |  | 6.19 | 377.00 |   |
| 107 | 0.605 |  | 7.24 | 499.25 |  |
| 108 | 1.14  |  | 6.45 | 514.45 |  |

|     |       |  |      |        |                                                                                       |
|-----|-------|--|------|--------|---------------------------------------------------------------------------------------|
| 109 | 0.893 |  | 6.44 | 510.30 |    |
| 110 | 0.049 |  | 7.64 | 445.20 |    |
| 111 | 0.349 |  | 5.73 | 470.00 |   |
| 112 | >10   |  | 8.07 | 432.00 |  |
| 113 | 0.111 |  | 8.55 | 468.00 |  |

|     |    |  |       |        |                                                                                       |
|-----|----|--|-------|--------|---------------------------------------------------------------------------------------|
| 114 | NT |  | 9.83  | 449.25 |    |
| 115 | NT |  | 7.66  | 484.30 |    |
| 116 | NT |  | 8.72  | 466.20 |   |
| 117 | NT |  | 8.70  | 466.20 |  |
| 118 | NT |  | 6.94  | 480.30 |  |
| 119 | NT |  | 10.72 | 468.20 |  |

\*\*All  $^1\text{H}$  NMR spectra were recorded on 400 MHz (Bruker) and 500 MHz (Agilent) NMR spectrometers. All chemical shifts are given as  $\delta$  value with reference to tetramethylsilane (TMS) as an internal standard.

Products were purified by flash chromatography on 100-200 mesh silica gel and final compound purified through preparative HPLC. The chemicals and solvents were purchased from industrial chemical suppliers and they were used without purification prior to use.

<sup>a</sup>Column: X-SELECT CSH C-18 (150 x 4.6 mm, 3.5  $\mu$ m); 5mM ammonium acetate + acetonitrile; 1.0 mL/min, Diluent: ACN: H<sub>2</sub>O).

## EXAMPLE 9

### [0347] Screening Compounds for Inhibition of BFT *In Vivo*

[0348] Test compounds were also screened *in vivo*. Germ-free (GF) mice were mono-colonized with ETBF on day 0. On days 1, 2, and 3 following colonization, 50 mg/kg of the test compound was orally administered to the mice once (QD) or two times per day (BID). Markers of injury and inflammation (e.g., cecal weight and fecal lipocalin 2) were analyzed on day 4 (FIG. 6A). Sample size was 5-6 mice per group.

[0349] As shown in FIG. 6B, administration of 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid (OH-CGS-23270A) significantly increased cecal weight in ETBF-colonized mice compared to the vehicle control, indicating that these test compounds significantly reduced cecal injury and inflammation associated with ETBF compared to the control. Fecal lipocalin2, a marker of intestinal inflammation, was significantly decreased in ETBF-colonized mice treated with 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid (OH-CGS-23270A) compared to the vehicle treated control (FIG. 6C).

[0350] Taken together, these data show that intestinal inflammation was reduced in mice colonized with ETBF upon treatment with 2(R)-[4-Hydroxy-N-(3-pyridylmethyl)phenylsulfonamido]-3-methylbutyrylhydroxamic acid. These data indicate that OH-CGS-23270A may be used to treat or prevent ETBF-mediated gastrointestinal disease in a subject, such as colitis or Crohn's disease. Compounds of the disclosure, Example 42, Example 60, and Example 113, significantly reduce lipocalin2 levels when dosed in the 15-50 mg/kg p.o. range, QD.

## EXAMPLE 10

### [0351] Administering BFT, ColA, and/or GeIE Inhibitors to a Subject

**[0352]** A compound capable of inhibiting BFT, ColA, and/or GelE is formulated as a tablet or capsule for oral administration. The pharmaceutical composition is administered to a subject in a therapeutically effective amount, i.e., an amount sufficient to inhibit BFT, ColA, and/or GelE in the subject.

#### **EXAMPLE 11**

##### **[0353] Treating a Subject that has IBD**

**[0354]** A subject having or suspected of having IBD is tested to determine if they have been colonized by an enterotoxigenic strain of one or more of *B. fragilis*, *E. faecalis*, or *C. perfringens*. If the subject tests positive for one or more of these bacteria or toxins produced thereby, a therapeutically effective amount of a compound capable of inhibiting BFT, GelE, and/or ColA is administered to the subject. The therapeutically effective amount is an amount sufficient to reduce the amount or the pathogenic effects of the one or more enterotoxigenic bacterial strains or toxins produced thereby. Disease progression in the subject is monitored. Subject stool samples may be tested to monitor the presence and/or abundance of the one or more pathogenic bacterial strains or toxins produced thereby, before and after administration of the compound.

#### **EXAMPLE 12**

##### **[0355] Screening Compounds for Inhibition of Gelatinase E**

**[0356]** Gelatinase purification

**[0357]** Gelatinase E (Gel E) was purified from bacterial culture supernatant from *E. faecalis*. *E. faecalis* was cultured aerobically in Todd Hewitt Broth overnight at 37 °C. Nucleic acid was precipitated with 0.9% protamine solution, followed by protein precipitation with ammonium sulfate. Resuspended protein pellet was further subjected to purification using FPLC (phenyl Sepharose column). Fractions with gelatinase activity as determined by casein agar assay were pooled and further concentrated.

**[0358]** Gelatinase E activity assay

**[0359]** Different concentrations of test compound were incubated with purified GelE and FRET-based peptide substrate (390 MMP FRET Substrate 1; Anaspec AS-27077) in assay buffer at room temperature for 30 minutes. The fluorescence signal was determined by a plate reader.

**[0360]** Various compounds in Table 10 inhibited GelE with an IC<sub>50</sub> of greater than 200  $\mu$ M. Compound 62 showed high levels of inhibition, with an IC<sub>50</sub> of about 16.42  $\mu$ M

**[0361]** Compound A also showed high levels of GelE inhibition as shown in FIG. 7 and Table 11 (below).

### EXAMPLE 13

#### **[0362] Screening Compounds for Inhibition of Collagenase H**

**[0363]** Collagenase H assay as a surrogate for ColA inhibition

**[0364]** Different concentrations of test compound were incubated with *Clostridium histolyticum* collagenase H (ColH) and fluorescein-labeled DQ-gelatin conjugate (both are components of EnzCheck Gelatinase/Collagenase Assay Kit, ThermoFisher E12055) at 37 °C for 2 hours. ColH has similar activity to ColA.

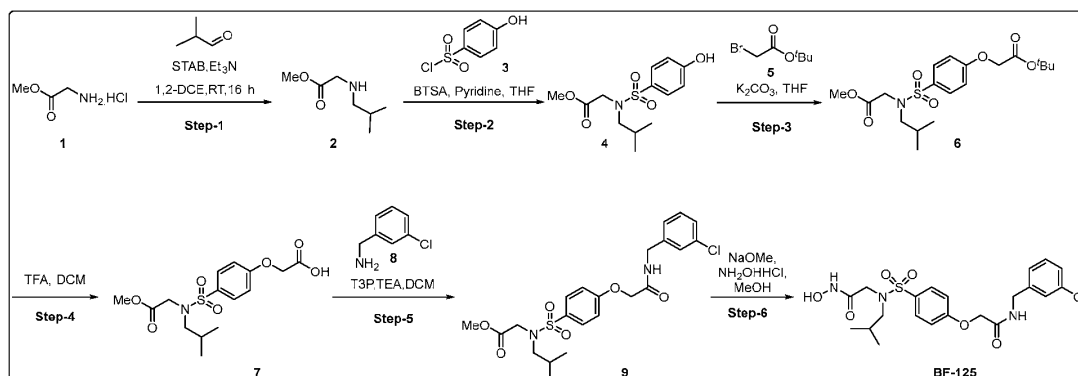
**[0365]** The fluorescence signal was determined by a plate reader and level of inhibition calculated.

**[0366]** Various compounds in Table 10 inhibited ColH, with IC<sub>50</sub> values of greater than 200  $\mu$ M or 400  $\mu$ M. The highest level of inhibition of ColH was observed for compounds 26 (IC<sub>50</sub> = 19.29  $\mu$ M), 33 (IC<sub>50</sub> = 0.384  $\mu$ M), 42 (IC<sub>50</sub> = 8.775  $\mu$ M), 60 (IC<sub>50</sub> = 3.825  $\mu$ M) and 63 (IC<sub>50</sub>=8.894  $\mu$ M).

**[0367]** Compound A also exhibited high levels of ColH inhibition, as shown in Table 11 (below).

### EXAMPLE 14

**[0368]** Synthesis of N-(3-chlorobenzyl)-2-(4-(N-(2-(hydroxyamino)-2-oxoethyl)-N-isobutyl sulfamoyl)phenolxy)acetamide (AG; BF-125)



**[0369] Step 1: Synthesis of methyl isobutylglycinate:**

**[0370]** To a stirred solution of methyl glycinate hydrochloride (1.5 g, 11.95 mmol) in Methanol (24 mL) were added isobutyraldehyde (0.86 g, 11.95 mmol) and Triethylamine (2.5 mL, 17.92 mmol) at RT; stirred for 1 h. Then AcOH (5.68 mL, 95.61 mmol) was added and maintained at RT for 16 h. The reaction mixture was heated at 50°C for 1 h, then cooled to rt and added NaBH<sub>3</sub>CN (monitored by TLC, after completion of the reaction, the volatiles were concentrated under reduced pressure, reaction mixture was diluted with water (10 mL) and extracted with EtOAc (2 x 100 mL), the combined organic extracts were washed with 6N HCl (10 mL). The aqueous layer was basified with Na<sub>2</sub>CO<sub>3</sub> and then extracted with EtOAc (2 x 100 mL), the combined organic extracts were washed with brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain the crude methyl isobutylglycinate (2) (1.1 g, crude) as light yellow liquid.

**[0371]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ = 3.63 - 3.59 (m, 2H), 3.30 (s, 3H), 2.28 (dd, *J* = 16.3, 7.0 Hz, 2H), 1.89 - 1.82 (m, 1H), 1.69 - 1.56 (m, 1H), 0.88 - 0.80 (m, 6H); LCMS: 90%, *m/z*: [M+H]<sup>+</sup>, 2.0 mass spec found, 146.0.

**[0372] Step 2: Synthesis of methyl N-((4-hydroxyphenyl)sulfonyl)-N-isobutylglycinate:**

**[0373]** To a stirred solution of 4-hydroxybenzenesulfonyl chloride (1.0 g, 5.20 mmol, 1.0 eq) in THF (5 mL) was added BTSA (1.05 g, 5.20 mmol, 1.0 eq) at 0 °C; stirred for 2.5 h. The above reaction mixture was added to a solution of methyl isobutylglycinate (5) (1.5 g, 5.20 mmol, 1.0 eq) in Pyridine (14 mL) at 0 °C, then stirred at RT for 20 h. The reaction was monitored by TLC, after completion of the reaction the solvent was evaporated. The crude was dissolved in EtOAc (120 mL), washed with water (3x 100 mL), 1N HCl (2x50

mL), water (2x100 mL) and brine solution (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain the crude. The crude product was purified by column chromatography over silica gel (100-200 mesh) using 20% EtOAc/DCM as eluent to obtain methyl N-((4-hydroxyphenyl)sulfonyl)-N-isobutylglycinate (4) (1.2 g, 76 %) as thick syrup.

**[0374]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ = 10.45 - 10.39 (m, 1H), 7.62 - 7.59 (m, 2H), 6.92 - 6.88 (m, 2H), 3.96 - 3.94 (m, 2H), 3.56 (s, 3H), 2.88 (d, *J* = 7.5 Hz, 2H), 1.75 (td, *J* = 13.5, 6.8 Hz, 1H), 0.81 (d, *J* = 6.6 Hz, 6H)

**[0375] Step 3: Synthesis of tert-butyl 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetate:** To a stirred solution of methyl N-((4-hydroxyphenyl)sulfonyl)-N-isobutylglycinate (0.2 g, 0.662 mmol) in DMF (3 mL) were added Cs<sub>2</sub>CO<sub>3</sub> (0.431 g, 1.32 mmol, 2 eq) and tert-butyl 2-bromoacetate (0.155 g, 0.79 mmol, 1.2 eq) at RT, then the reaction mixture was stirred at RT for 3 h. The reaction was monitored by TLC, after completion of the starting material, reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 x 10 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to obtain the tert-butyl 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetate (0.25 g) as a crude compound. The crude compound was directly used for the next step without further purification.

**[0376]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ = 7.76 - 7.72 (m, 2H), 7.11 - 7.07 (m, 2H), 4.80 (s, 2H), 4.01 (s, 2H), 3.56 (s, 3H), 2.93 (d, *J* = 7.5 Hz, 2H), 1.84 - 1.73 (m, 1H), 1.44 (s, 9H), 0.84 - 0.80 (m, 6H).

**[0377] Step 4: Synthesis of 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetic acid:** To a stirred solution of tert-butyl 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetate (0.1 g, 0.24 mmol) in DCM (2 mL) was added TFA (2 mL) at 0 °C; then the reaction mixture was stirred at RT for 16 h. The reaction was monitored by TLC, after completion of the starting material, reaction mixture was concentrated to dryness to afford 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetic acid (0.1 g) as a crude compound and directly used for the next step without further purification.

**[0378]** <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ = 13.19 - 13.07 (m, 1H), 7.72 (br d, *J* = 8.8 Hz, 2H), 7.08 (br d, *J* = 8.8 Hz, 2H), 4.80 (s, 2H), 4.02 - 3.96 (m, 2H), 3.54 (s, 3H), 2.90 (br d,

$J = 7.3$  Hz, 2H), 1.79 - 1.73 (m, 1H), 0.82 (br d,  $J = 6.4$  Hz, 6H); LCMS: 92.7%,  $m/z$ :  $[M+H]^+$ , mass spec calculated for  $C_{15}H_{21}NO_7S$ , 359.39; mass spec found, 360.1.

**[0379] Step 5: Synthesis of methyl N-((4-(2-((3-chlorobenzyl)amino)-2-oxoethoxy)phenyl)sulfonyl)-N-isobutyl glycinate:** To a stirred solution of 2-(4-(N-isobutyl-N-(2-methoxy-2-oxoethyl)sulfamoyl)phenoxy)acetic acid (250 mg, 0.69 mmol) in DCM (6 mL) were added Triethylamine (209 mg, 2.07 mmol) and  $T_3P$  (0.65 mL, 1.03 mmol) at 0 °C; then the reaction mixture was stirred at RT for 30 min. then added 3-chloromethanamine (0.044 gm, 0.361 mmol) at 0 °C. The reaction mixture was stirred at RT for 3 h. The reaction progress was monitored by TLC. After completion of reaction, reaction mixture was concentrated to dryness and the crude compound was purified by silica gel column chromatography, eluted with 60% Ethyl acetate in n-Heptane to afforded methyl N-((4-(2-((3-chlorobenzyl)amino)-2-oxoethoxy)phenyl)sulfonyl)-N-isobutylglycinate (170 mg, Yield = 50%) as a off white solid.

**[0380] LCMS:** 87.36%,  $m/z$ :  $[M]^+$ ; mass spec calculated for  $C_{22}H_{27}ClN_2O_6S$ , 482.98; mass spec found, 483.1.

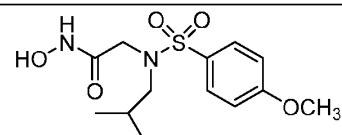
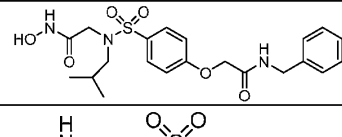
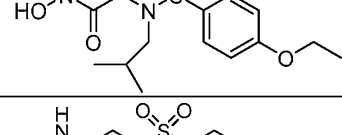
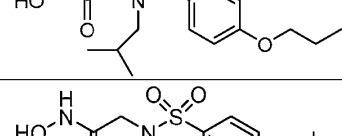
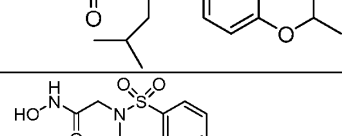
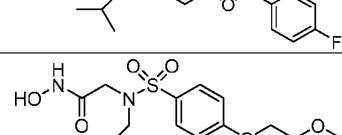
**[0381] Step 6: Synthesis of N-(3-chlorobenzyl)-2-(4-(N-(2-(hydroxyamino)-2-oxoethyl)-N-isobutylsulfamoyl) phenoxy)acetamide (AG):** To a stirred solution of methyl N-((4-(2-((3-chlorobenzyl)amino)-2-oxoethoxy)phenyl)sulfonyl)-N-isobutylglycinate (85 mg, 0.17 mmol) in MeOH (5 mL) were added Sodium methoxide (137 mg, 2.55 mmol, 15 eq) and Hydroxylamine hydrochloride (0.360 g, 5.19 mmol, 15 eq); then the reaction mixture was heated at 58 °C for 7.5 h. The progress of the reaction was monitored by TLC. After completion of the reaction, reaction mixture was concentrated under reduced pressure; added IPA (20 mL), insoluble material was removed by filtration and filtrate was concentrated under reduced pressure to obtain the crude. The crude product was purified by prep HPLC to obtain N-hydroxy-2-((N-isobutyl-4-(2-((4-methylbenzyl)amino)-2-oxoethoxy)phenyl)sulfonamido)acetamide (**AG; BF-125**; 17 mg, yield=20%) as an off-white semi-solid.

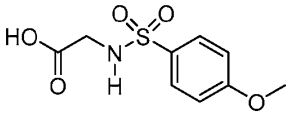
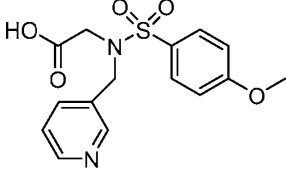
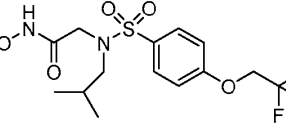
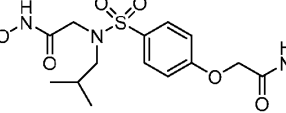
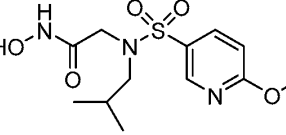
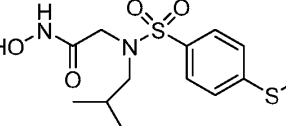
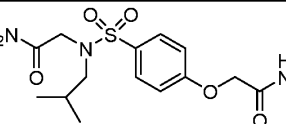
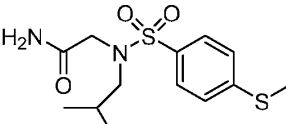
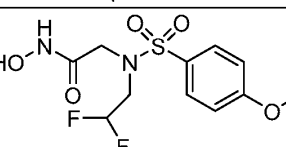
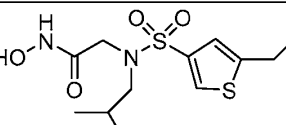
**[0382]**  $^1H$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  = 10.67 - 10.39 (m, 1H), 8.94 - 8.84 (m, 1H), 8.82 - 8.65 (m, 1H), 7.84 - 7.69 (m, 2H), 7.40 - 7.28 (m, 3H), 7.22 (br d,  $J = 7.0$  Hz, 1H), 7.12 (br d,  $J = 8.3$  Hz, 2H), 4.69 (br s, 2H), 4.35 (br d,  $J = 5.5$  Hz, 2H), 3.76 - 3.52 (m, 2H), 3.00 - 2.78 (m, 2H), 1.91 - 1.74 (m, 1H), 0.81 (br d,  $J = 6.3$  Hz, 6H)

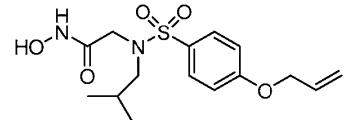
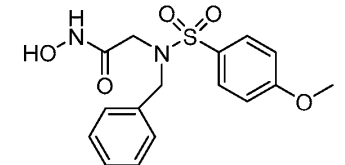
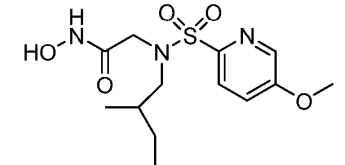
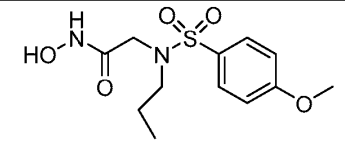
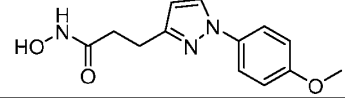
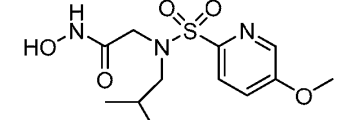
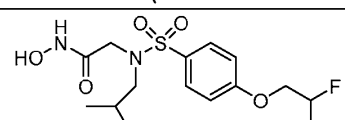
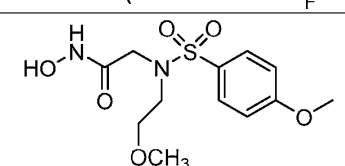
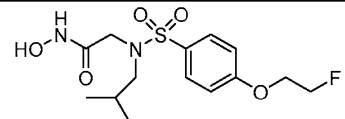
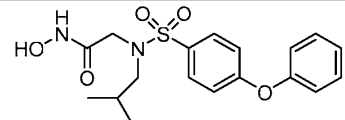
**[0383] LCMS:** 99.45%, m/z: [M+H]<sup>+</sup>; mass spec calculated for C<sub>21</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>6</sub>S, 483.96; mass spec found, 484.2. (Column; Kinetex EVO C18 (3.0 x 50 mm, 2.6 μm); RT: 1.96 min; A: 0.5 ml Formic acid in 950 ml water + 50 ml ACN, B: 0.5 ml Formic acid in ACN; Gradient program: 0.01/2, 0.2/2, 2.2/98, 3/98, 3.2/2, 4/2; Flow: 1.2 mL/min.

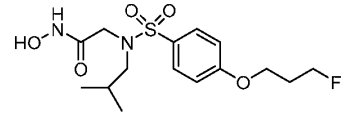
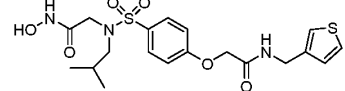
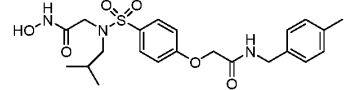
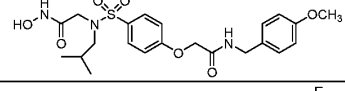
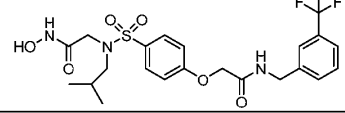
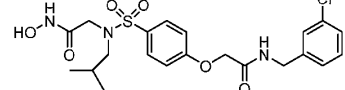
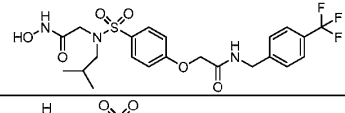
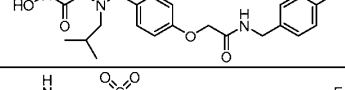
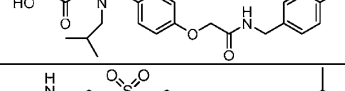
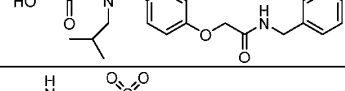
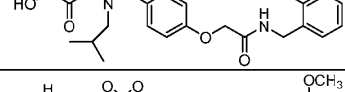
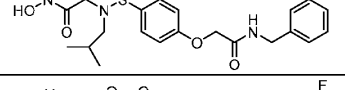
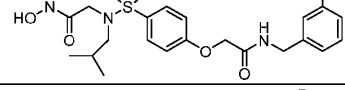
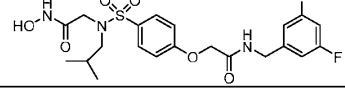
**[0384] HPLC:** 99.68%; (Column; X-SELECT CSH C-18 (4.6 x 150 mm, 3.5 μm); RT: 9.17 min; A: 0.05% TFA: ACN (95:05), B: ACN: 0.05% TFA (95:05); T/B%: 0.01/10, 12/90, 16/90; Flow: 1.0 mL/min.

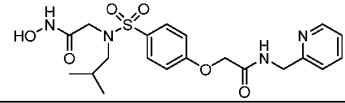
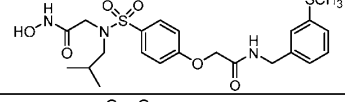
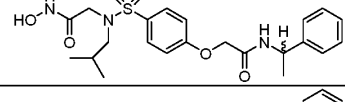
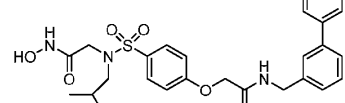
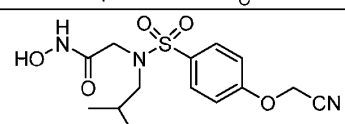
**Table 11.** Collagenase H and Gelatinase E Inhibition by Compounds of the Present Disclosure

| Cmpd. #* | ColH IC50 (μM) | GelE IC50 (μM) | HPLC Retention Time (Min) <sup>a</sup> | LC-MS (M+H) | Structure                                                                            |
|----------|----------------|----------------|----------------------------------------|-------------|--------------------------------------------------------------------------------------|
| A        | 0.612          | 0.202          | 8.11                                   | 317.10      |   |
| B        |                | 0.130          | 8.31                                   | 450.20      |  |
| C        | >100           | 0.372          | 7.29                                   | 331.10      |  |
| D        | >100           | 0.489          | 9.53                                   | 345.10      |  |
| E        | >100           | 1.21           | 8.03                                   | 345.00      |  |
| F        | >100           | 0.649          | 10.37                                  | 411.00      |  |
| G        | >100           | 0.443          | 7.36                                   | 361.00      |  |

|   |      |       |       |        |                                                                                       |
|---|------|-------|-------|--------|---------------------------------------------------------------------------------------|
| H | >100 | >100  | 5.90  | 244.00 |    |
| I | >100 | >100  | 2.91  | 337.10 |    |
| J | >100 | 0.387 | 9.26  | 383.10 |    |
| K | >100 | 0.512 | 4.58  | 374.00 |    |
| L | >100 | 0.336 | 7.16  | 318.10 |   |
| M | >100 | 0.257 | 8.57  | 333.10 |  |
| N | >100 | >100  | 7.32  | 359.00 |  |
| O | >100 | >100  | 10.44 | 318.00 |  |
| P | >100 | 9.09  | 6.63  | 325.00 |  |
| Q | NT   | 0.733 | 8.70  | 319.20 |  |

|           |    |        |       |        |                                                                                      |
|-----------|----|--------|-------|--------|--------------------------------------------------------------------------------------|
| <b>R</b>  | NT | 2.419  | 8.96  | 343.20 |    |
| <b>S</b>  | NT | 0.2045 | 8.02  | 351.20 |    |
| <b>T</b>  | NT | 0.290  | 8.31  | 331.20 |    |
| <b>U</b>  | NT | 8.531  | 6.79  | 301.10 |    |
| <b>V</b>  | NT | 12.71  | 6.05  | 262.20 |   |
| <b>W</b>  | NT | 0.299  | 6.06  | 318.10 |  |
| <b>X</b>  | NT | 0.180  | 8.42  | 367.00 |  |
| <b>Y</b>  | NT | 6.765  | 7.91  | 318.95 |  |
| <b>Z</b>  | NT | 0.246  | 7.93  | 349.20 |  |
| <b>AA</b> | NT | 1.69   | 10.22 | 379.20 |  |

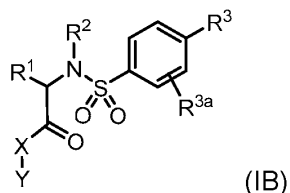
|           |    |       |      |        |                                                                                      |
|-----------|----|-------|------|--------|--------------------------------------------------------------------------------------|
| <b>AB</b> | NT | 2.40  | 7.60 | 363.10 |    |
| <b>AC</b> | NT | 0.139 | 8.08 | 456.10 |    |
| <b>AD</b> | NT | 0.155 | 8.00 | 464.20 |    |
| <b>AE</b> | NT | 0.156 | 8.32 | 480.20 |    |
| <b>AF</b> | NT | 0.113 | 9.67 | 518.20 |    |
| <b>AG</b> | NT | 0.103 | 9.17 | 484.20 |    |
| <b>AH</b> | NT | 0.238 | 9.68 | 518.20 |   |
| <b>AI</b> | NT | 0.164 | 9.26 | 484.10 |  |
| <b>AJ</b> | NT | 0.313 | 8.61 | 468.20 |  |
| <b>AK</b> | NT | >10   | 8.80 | 464.20 |  |
| <b>AL</b> | NT | 0.218 | 8.81 | 464.20 |  |
| <b>AM</b> | NT | 0.118 | 8.48 | 478.20 |  |
| <b>AN</b> | NT | 0.158 | 6.81 | 468.20 |  |
| <b>AO</b> | NT | >10   | 8.82 | 486.20 |  |

|           |    |       |       |        |                                                                                    |
|-----------|----|-------|-------|--------|------------------------------------------------------------------------------------|
| <b>AP</b> | NT | 0.177 | 4.69  | 451.20 |  |
| <b>AQ</b> | NT | 0.119 | 7.04  | 496.20 |  |
| <b>AR</b> | NT | 0.237 | 6.89  | 464.00 |  |
| <b>AS</b> | NT | NT    | 10.30 | 526.20 |  |
| <b>AT</b> | NT | NT    | 6.94  | 342.15 |  |

## CLAIMS

What is claimed is:

1. A compound of Formula IB:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-;

Y is -OH;

R<sup>1</sup> is alkyl, haloalkyl, -alkylene-OH, -alkylene-NH<sub>2</sub>, -alkylene-C(=O)NH<sub>2</sub>, heteroaralkyl, aryl, aralkyl, -alkylene-S-alkyl, -alkylene-S-haloalkyl, -alkylene-S-aralkyl, or -alkylene-S-heteroaralkyl; wherein R<sup>1</sup> is optionally substituted with one or more groups selected from -OH, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>;

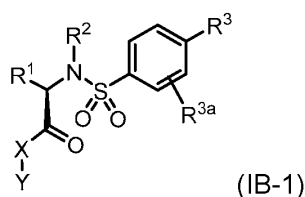
R<sup>2</sup> is H, -(CH<sub>2</sub>)<sub>n</sub>-aryl, -CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -CH<sub>2</sub>-heterocyclyl, -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or -CH<sub>2</sub>-haloalkyl;

R<sup>3</sup> is -OH;

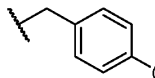
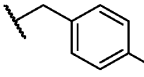
R<sup>3a</sup> is H or halogen; and

n is an integer from 1-3.

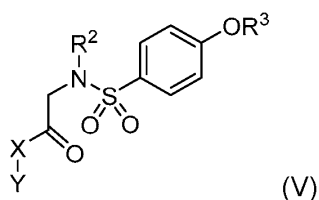
2. The compound of claim 1, wherein the compound of Formula IB has the structure of Formula IB-1:



or a stereoisomer or a pharmaceutically acceptable salt thereof.

3. The compound of claim 1 or 2, wherein R<sup>1</sup> is -C<sub>1</sub>-C<sub>6</sub> alkyl, -C<sub>1</sub>-C<sub>6</sub> alkyl-OH, -(C<sub>1</sub>-C<sub>3</sub> alkylene)-S-(C<sub>1</sub>-C<sub>3</sub> alkyl), -(C<sub>1</sub>-C<sub>3</sub> alkylene)-S-(C<sub>1</sub>-C<sub>3</sub> haloalkyl), -(C<sub>1</sub>-C<sub>3</sub> alkylene)-SCH<sub>2</sub>-heteroaryl, -CH<sub>2</sub>-phenyl, -CH<sub>2</sub>-heteroaryl, or -CH<sub>2</sub>C(=O)NH<sub>2</sub>, wherein phenyl is optionally substituted with one or more groups selected from -OH, -OMe, halogen, -CHF<sub>2</sub>, -CH<sub>2</sub>F, or -CF<sub>3</sub>.
4. The compound of claim 1 or 2, wherein R<sup>1</sup> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH(OH)CH<sub>3</sub>, -CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>, , -CH<sub>2</sub>-phenyl, -CH<sub>2</sub>-(3-indolyl), -CH<sub>2</sub>-(4-imidazolyl), -CH<sub>2</sub>C(=O)NH<sub>2</sub>, , -CH(CH<sub>3</sub>)SCH<sub>2</sub>CH<sub>3</sub>, -CH(CH<sub>3</sub>)SCH<sub>2</sub>-(3-pyridyl), -CH(CH<sub>3</sub>)SCH<sub>2</sub>-(4-pyridyl), or -CH(CH<sub>3</sub>)SCH<sub>2</sub>CF<sub>3</sub>.
5. The compound of claim 1 or 2, wherein R<sup>1</sup> is alkyl, haloalkyl, -alkylene-OH, alkylene-O-alkyl, -alkylene-S-alkyl, heteroaralkyl, aryl, or aralkyl.
6. The compound of claim 1 or 2, wherein R<sup>1</sup> is alkyl, -alkylene-OH, alkylene-O-alkyl, heteroaralkyl, aryl, or aralkyl.
7. The compound of claim 1 or 2, wherein R<sup>1</sup> is alkyl, aryl, -alkylene-OH, alkylene-O-alkyl.
8. The compound of claim 1 or 2, wherein R<sup>1</sup> is alkyl or aryl.
9. The compound of claim 1 or 2, wherein R<sup>1</sup> is alkyl.
10. The compound of any one of claims 5-9, wherein the alkyl is a C<sub>2-6</sub> alkyl.
11. The compound of any one of claims 5-10, wherein the alkyl is ethyl or isobutyl.
12. The compound of any one of claims 5-10, wherein the alkyl is ethyl.
13. The compound of any one of claims 5-8, wherein the aryl is a C<sub>6-12</sub> aryl.
14. The compound of claim 13, wherein the C<sub>6-12</sub> aryl is phenyl.

15. The compound of claim 14, wherein the phenyl is substituted with one or more halogens.
16. The compound of claim 13 or 14, wherein the phenyl is 4-fluorophenyl.
17. The compound of any one of claims 5-7, wherein the alkylene is a C<sub>1-3</sub>alkylene.
18. The compound of any one of claims 5-7, wherein the alkylene is a methylene.
19. The compound of any one of claims 1-18, wherein R<sup>2</sup> is -CH<sub>2</sub>-alkyl, -(CH<sub>2</sub>)<sub>n</sub>-aryl, or -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl.
20. The compound of any one of claims 1-19, wherein R<sup>2</sup> is -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl.
21. The compound of claim 19 or 20, wherein the heteroaryl is a 5- to 14-membered heteroaryl having 1, 2, or 3 heteroatoms selected from N, O, or S.
22. The compound of claim 20 or 21, wherein the heteroaryl is pyridyl, thiophenyl, thiazoyl, oxazolyl, or indolyl.
23. The compound of any one of claims 20-22, wherein the heteroaryl is pyridyl or indolyl.
24. The compound of one of claims 20-23, wherein the heteroaryl is 3-pyridyl or 5-indolyl.
25. The compound of any one of claims 1-24, wherein n is 1 or 2.
26. A compound of Formula V:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

X is -NH-;

Y is -OH-;

R<sup>2</sup> is -(CH<sub>2</sub>)<sub>n</sub>-aryl, -CH<sub>2</sub>-alkyl, -CH(Me)-alkyl, -(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, or -CH<sub>2</sub>-haloalkyl;

and

R<sup>3</sup> is H, alkyl, -alkylene-NR<sup>5</sup>R<sup>6</sup>, haloalkyl, aryl, aralkyl, or heteroaryl, each of which is optionally substituted;

R<sup>5</sup> is H, alkyl, aralkyl, heteroaralkyl, -C(O)alkyl, -C(O)aryl, -C(O)heteroaryl, or -C(O)aralkyl;

R<sup>6</sup> is H, alkyl, or aryl; and

n is an integer from 1-3.

27. The compound of claim 26, wherein R<sup>2</sup> is -CH<sub>2</sub>alkyl.

28. The compound of claim 26 or 27, wherein R<sup>2</sup> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -CH<sub>2</sub>CH(CH<sub>3</sub>)(CH<sub>2</sub>CH<sub>3</sub>), -CH<sub>2</sub>CH<sub>2</sub>OCH<sub>3</sub>, or -CH<sub>2</sub>CHF<sub>2</sub>.

29. The compound of any one of claims 26-28, wherein R<sup>2</sup> is -CH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>.

30. The compound of any one of claims 26, wherein R<sup>2</sup> is -CH<sub>2</sub>-aryl, -CH<sub>2</sub>-alkyl, -or -CH<sub>2</sub>-heteroaryl.

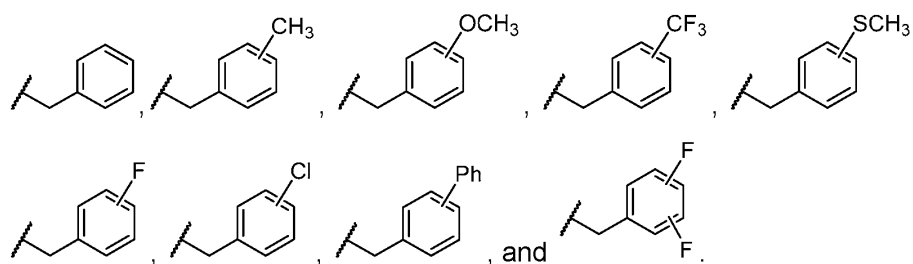
31. The compound of claim 26 or 30, wherein R<sup>2</sup> is -CH<sub>2</sub>-Ph, -CH<sub>2</sub>-CH(CH<sub>3</sub>)<sub>2</sub>, -or -CH<sub>2</sub>-(3-pyridyl).

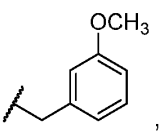
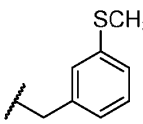
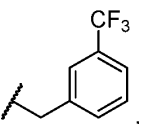
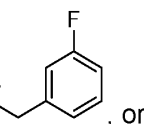
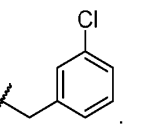
32. The compound of any one of claims 26-31, wherein R<sup>3</sup> is -OH, alkoxy, -O-haloalkyl, -O-aralkyl, -O-heteroaralkyl, -O-alkylene-NR<sup>5</sup>R<sup>6</sup>, alkyl or -N(H)C(O)-alkylene-NR<sup>5</sup>R<sup>6</sup>, wherein the alkylene is optionally substituted with F, oxo, alkyl, fluoroalkyl, aryl, -CH<sub>2</sub>-aryl, or -CH<sub>2</sub>-heteroaryl.

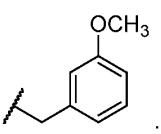
33. The compound of any one of claims 26-32, wherein R<sup>3</sup> is -OH, alkoxy, or -O-alkylene-NR<sup>5</sup>R<sup>6</sup>.

34. The compound of any one of claims 26-33, wherein the alkoxy is -OCH<sub>3</sub>.

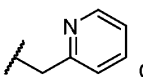
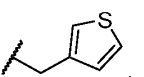
35. The compound of any one of claims 26-35, wherein the alkylene is a C<sub>1-3</sub> alkylene.
36. The compound of any one of claims 26-35, wherein the alkylene is a methylene or ethylene.
37. The compound of claim 36, wherein the -O-alkylene-NR<sup>5</sup>R<sup>6</sup> is -O-CH<sub>2</sub>-C(O)-NR<sup>5</sup>R<sup>6</sup>.
38. The compound of any one of claims 26-37, wherein R<sup>5</sup> is H or aralkyl or heteroaralkyl.
39. The compound of claim 26-38, wherein the aralkyl is -CH<sub>2</sub>aryl.
40. The compound of claim 26-38, wherein the aralkyl is -CH<sub>2</sub>Ph.
41. The compound of claim 40, wherein the phenyl is optionally substituted with one or more halogen, alkyl, haloalkyl, alkoxy, thioalkyl, aryl, heteroaryl or combinations thereof.
42. The compound of any one of claims 38-41, wherein aralkyl is selected from the group consisting of:



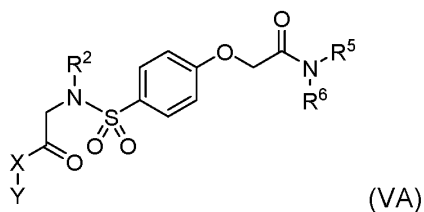
43. The compound of any one of claims 38-42, wherein the aralkyl is , , , , or .

44. The compound of any one of claims 38-43, wherein the aralkyl is .

45. The compound of any one of claims 38-44, wherein the heteroaralkyl is -CH<sub>2</sub>pyridyl or -CH<sub>2</sub>thiophenyl.

46. The compound of any one of claims 38-45, wherein the heteroaralkyl is  or .

47. The compound of any one of claims 26-46, wherein R<sup>6</sup> is H or alkyl.
48. The compound of claim 47, wherein the alkyl is a C<sub>1-5</sub> alkyl.
49. The compound of any one of claims 26-48, wherein n is 1 or 2.
50. The compound of any one of claims 26-48, wherein n is 1.
51. The compound of claim 26, wherein the compound of Formula V has a structure according to:



or a stereoisomer or a pharmaceutically acceptable salt thereof, wherein:

$R^2$  is alkyl,  $-(CH_2)_n$ -aryl, or  $-(CH_2)_n$ -heteroaryl;

$R^5$  is H, aralkyl or heteroaralkyl;

$R^6$  is H, alkyl, or aryl; and

$n$  is an integer from 0-3.

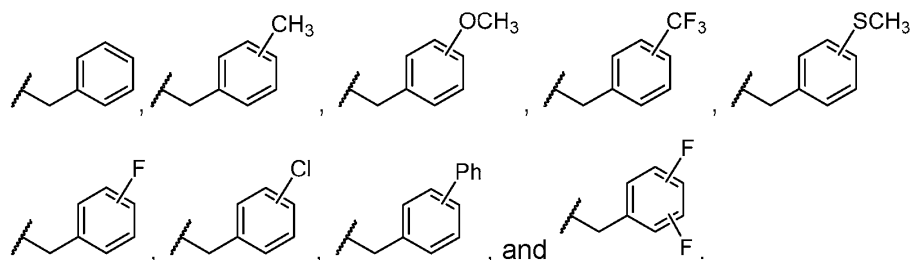
52. The compound of claim 51, wherein  $R^2$  is a  $C_{1-5}$  alkyl,  $-CH_2Ph$  or  $-CH_2pyridyl$ .

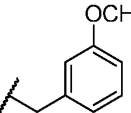
53. The compound of claim 51 or 52, wherein  $R^2$  is  $C_{1-5}$  alkyl.

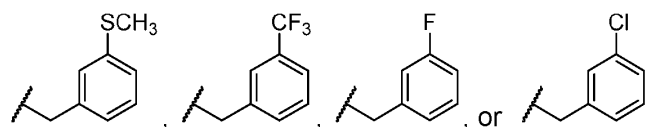
54. The compound of any one of claims 51-53, wherein  $R^2$  is  $-CH_2CH(CH_3)_2$ ,  $-CH_2CH_2CH_3$ ,  $-CH_2CH(CH_3)(CH_2CH_3)$ ,  $-CH_2CH_2OCH_3$ , or  $-CH_2CHF_2$ .

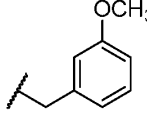
55. The compound of any one of claims 51, wherein  $R^5$  is  $-CH_2aryl$  or  $-CH_2heteroaryl$ .

56. The compound of claim 55, wherein the  $-CH_2aryl$  is selected from the group consisting of:



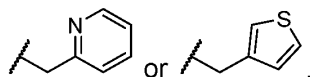
57. The compound of claim 55 or 56, wherein the  $-CH_2aryl$  is .



58. The compound of any one of claims 55-57, wherein the  $-CH_2aryl$  is .

59. The compound of any one of claims 55-58, wherein the  $-\text{CH}_2\text{heteroaryl}$  is  $-\text{CH}_2\text{pyridyl}$  or  $-\text{CH}_2\text{thiophenyl}$ .

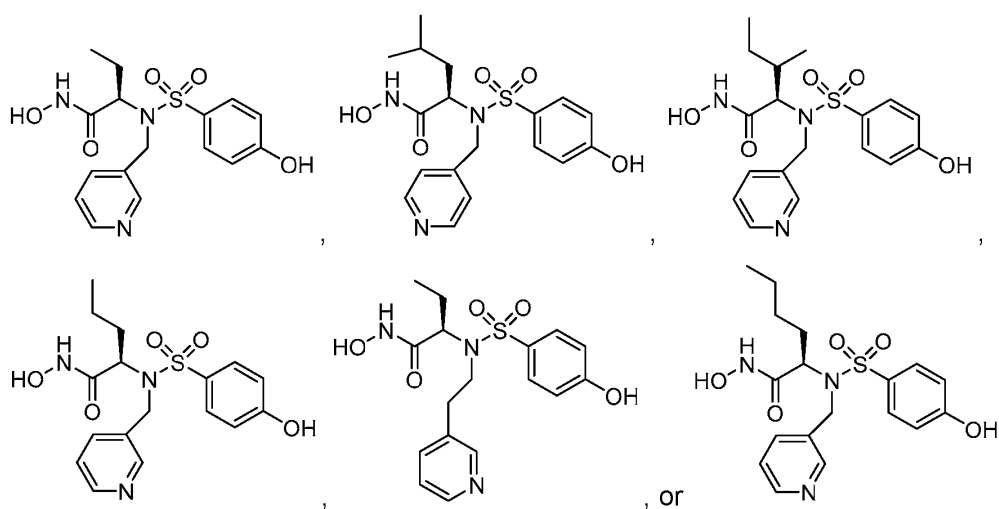
60. The compound of any one of claims 55-59, wherein the  $-\text{CH}_2\text{heteroaryl}$  is



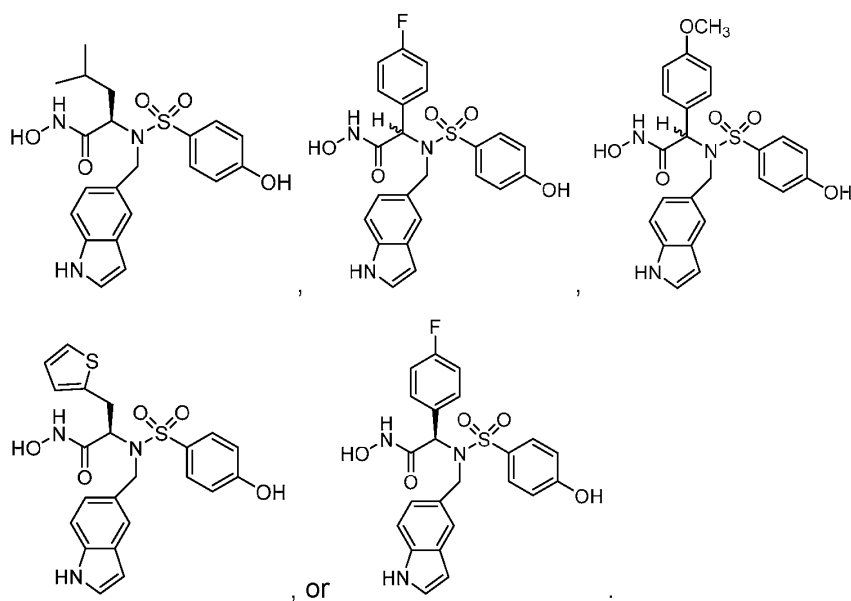
61. The compound of any one of claims 51-60, wherein  $\text{R}^6$  is H.

62. The compound of any one of claims 51-61, wherein  $n$  is 1 or 2.

63. The compound of claim 1, wherein the compound of Formula I has a structure according to:

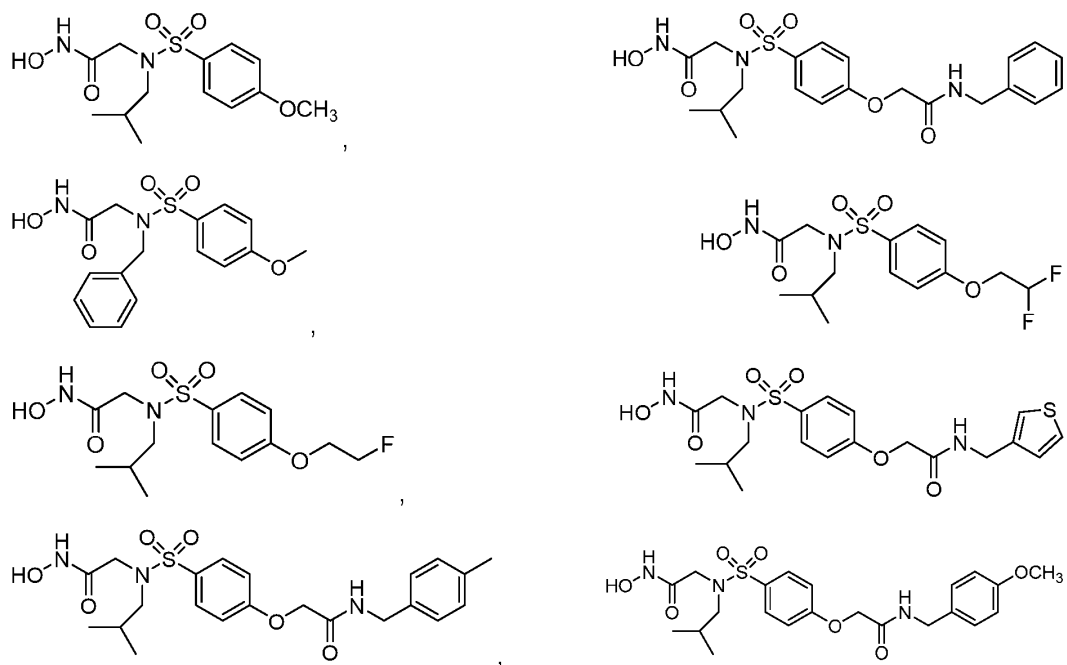


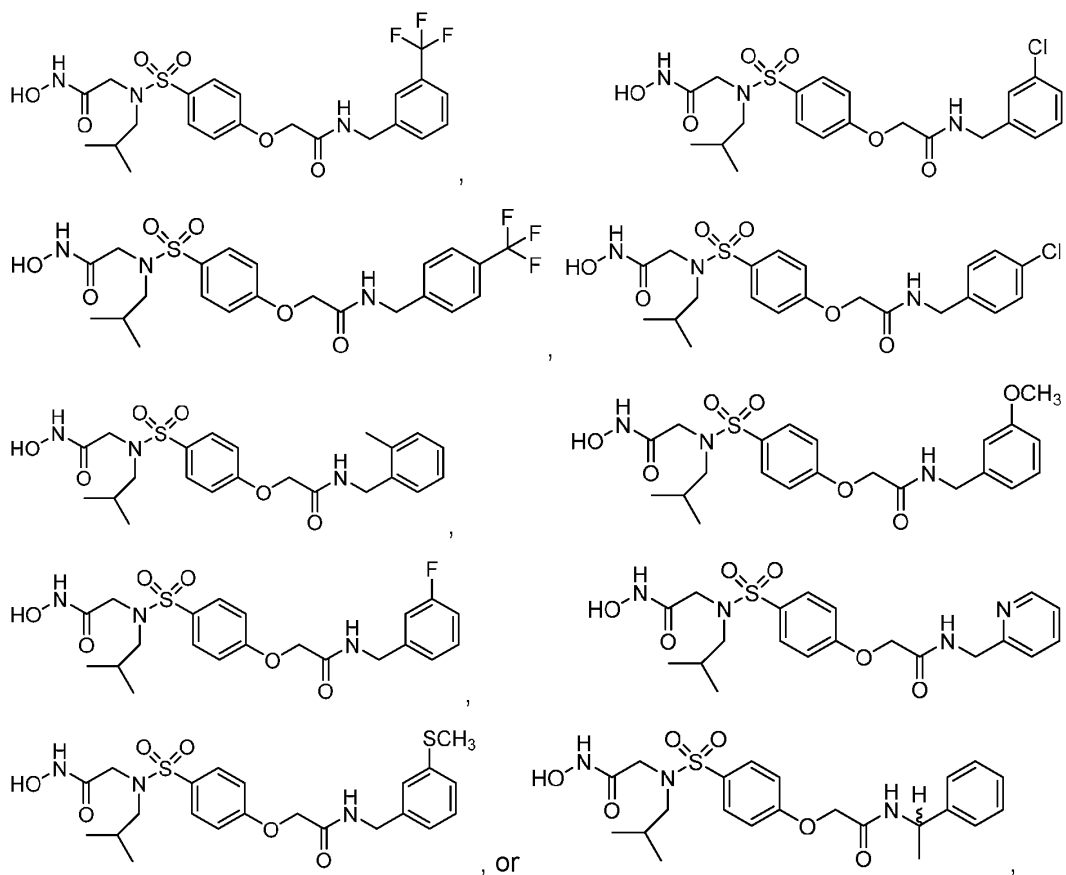
64. The compound of claim 1, wherein the compound of Formula I has a structure according to:



or a stereoisomer or a pharmaceutically acceptable salt thereof.

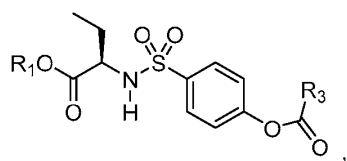
65. The compound of claim 29, wherein the compound has a structure according to:





or a stereoisomer or a pharmaceutically acceptable salt thereof.

66. A compound of Formula A:



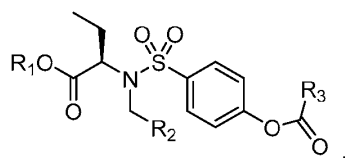
wherein:

R<sub>1</sub> is H or Me;

R<sub>3</sub> is Me or t-Bu; and

R<sub>4</sub> is H, Me, or Et.

67. A Compound of Formula B:



wherein:

R<sub>1</sub> is H or Me;

R<sub>2</sub> is Ph, 3-Pyr, -CH<sub>2</sub>Ph, or -CH<sub>2</sub>-3-Pyr;

R<sub>3</sub> is Me or t-Bu; and

R<sub>4</sub> is H, Me, or Et.

68. A pharmaceutical composition comprising a compound of any one of claims 1-65 and a pharmaceutically acceptable carrier or excipient.

69. A method of treating inflammatory bowel disease in a subject in need thereof, the method comprising, administering to the subject a therapeutically effective amount of a compound of any one of claims 1-65.

70. The method of claim 69, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.

71. A method of treating gastrointestinal (GI) cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-65.

72. The method of claim 71, wherein the GI cancer is esophageal cancer, gallbladder cancer, liver cancer, pancreatic cancer, stomach cancer, cancer of the small intestine, colorectal cancer, or anal cancer.

73. A method of treating a systemic bacterial infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1-65.

74. The method of claim 73, wherein the systemic bacterial infection is endocarditis or a urinary tract infection.

75. The method of any one of claims 69-74, wherein the subject is colonized by one or more pathogenic bacterial strain.

76. The method of claim 75, wherein the pathogenic bacterial strain is *B. fragilis*, *E. faecalis*, and/or *C. perfringens*.

77. The method of claim 75 or 76, wherein the pathogenic bacterial strain is a strain of *B. fragilis* expressing the BFT toxin, a strain of *E. faecalis* expressing the gelatinase GelE, or a strain of *C. perfringens* expressing the collagenase ColA.

78. The method of any one of claims 69-77, wherein the compound binds to and/or inhibits one or more of *B. fragilis* toxin (BFT), collagenase A (ColA), and gelatinase E (GelE).

79. The method of claim 78, wherein the BFT comprises the amino acid sequence of any one of SEQ ID NO: 2-4.

80. The method of claim 78, wherein the BFT comprises an amino acid sequence that is at least 98% identical to any one of SEQ ID NO: 2-4.

81. The method of claim 78, wherein the ColA comprises the amino acid sequence of SEQ ID NO: 8.

82. The method of claim 78, wherein the ColA comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 8.

83. The method of claim 79, wherein the GelE comprises the amino acid sequence of SEQ ID NO: 6.

84. The method of claim 80, wherein the GelE comprises an amino acid sequence that is at least 98% identical to SEQ ID NO: 6.

85. The method of any one of claims 77-84, wherein the compound binds to BFT, ColA, and/or GelE with an inhibition constant in the range of about  $10^{-5}$  to about  $10^{-13}$  M.

86. The method of any one of claims 77-84, wherein the compound has an IC<sub>50</sub> in the range of about 1  $\mu$ M to about 500  $\mu$ M.
87. The method of claim 86, wherein the IC<sub>50</sub> is determined by measuring cleavage of a FRET-based peptide substrate.
88. The method of claim 86, wherein the FRET-based peptide substrate has a sequence of SEQ ID NO: 10.
89. The method of any one of the claims 77-89, wherein administering the compound reduces and/or eliminates the activity of at least one of BFT, ColA and/or GelE.
90. The method of any one of claims 69-89, wherein the subject is a mammal.
91. The method of any one of claims 69-90, wherein the subject is a human.
92. The method of claim 91, wherein the subject is male.
93. The method of claim 91, wherein the subject is female.
94. The method of any one of claims 69-93, wherein the compound is administered intravenously to the subject.
95. The method of any one of claims 69-93, wherein the compound is administered orally to the subject.
96. The method of claim 95, wherein the compound is administered in a tablet or a capsule.
97. The method of claim 96, wherein the tablet or capsule comprises a pharmaceutically acceptable carrier or excipient.
98. The method of any one of claims 69-95, wherein the compound is administered as a liquid formulation.
99. The method of claim 98, wherein the liquid formulation comprises a pharmaceutically acceptable carrier or excipient.

100. The method of any one of claims 69-99, wherein the compound is administered once per day, once per week, or multiple times per day or week.

101. The method of any one of claims 69-100, wherein a dose of the compound administered to the subject is from about 0.001 to about 1000 mg/kg of body weight per day.