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(54) **Title:** SANTACRUZAMATE A COMPOSITIONS AND ANALOGS AND METHODS OF USE

(57) **Abstract:** The compositions and methods described herein relate generally to Santacruzamate A compositions and analogs, which, among other features, are useful as histone deacetylase (HDAC) inhibitors.



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Santacruzamate A Compositions and Analogs and Methods of Use

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

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5 TW008002 awarded by the National Institutes of Health. The government has certain rights
in this invention.

FIELD

The present disclosure relates generally to Santacruzamate A compositions and
analogs, which, among other features, are useful as histone deacetylase (HDAC) inhibitors.

10

BACKGROUND

Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes
involved in regulating the packaging of DNA around histones and thus have substantial
effects on gene transcription. HDAC inhibition prevents the removal of acetyl groups from
15 histones, leaving them unable to package DNA and causing their accumulation in cell nuclei.
This results in several downstream effects, many of which have significant importance in
causing apoptosis, differentiation, and/or reduced cell proliferation in cancer cells and may
have relevance to other diseases as well.

HDAC inhibitors are an emerging class of drugs that have generated considerable interest
20 as potential treatments for cancer, infectious disease, Alzheimer's disease and inflammation.

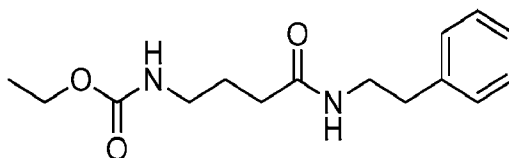
The first HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA, trade name
VORINOSTAT), was approved for clinical use in 2006 in patients with refractory cutaneous
T-cell lymphoma. In 2009, another HDAC inhibitor was also approved for cutaneous T-cell
lymphoma (romidepsin, trade name ISTODAX).

25 In humans, there are four classes of HDAC enzymes including Class I (HDAC 1-3,
8), Class IIa (HDAC 4, 5, 7, 9), Class IIB (HDAC 6, 10), Class III (sirtuins 1-7), and Class
IV (HDAC 11). Both HDAC inhibitors in clinical use, SAHA and romidepsin, are
considered pan-HDAC inhibitors with activity across several classes of HDAC enzyme,
resulting in considerable side effects upon treatment of subjects with these agents.

30 There remains a strong need for isozyme selective HDAC inhibitors that have potent
activity against one class of HDAC enzymes with little or no effect on the other classes. Such
selective HDAC inhibitors will result in increased clinical utility and decreased side effects.

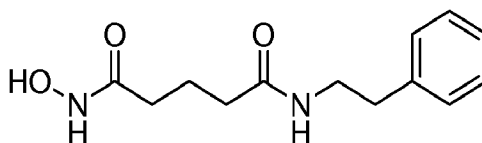
SUMMARY

Bioactivity-guided fractionation of an extract derived from a dark brown cyanobacterium isolated during an expedition to the Coiba National Park on the Pacific coast of Panama led to the isolation of the new compound, designated Santacruzamate A (SCA).
 5 Provided herein are compositions and analogs of a new compound, designated Santacruzamate A. Also provided are methods for their use in inhibiting HDAC and in treating HDAC-related diseases (e.g., cancer and/or neurological disorders). A compound described herein includes Santacruzamate A:



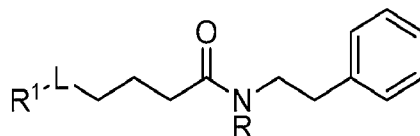
10 and pharmaceutically acceptable salts and prodrugs thereof. Optionally, the compound is synthetic Santacruzamate A.

A further compound described herein includes the compound having the following structure:

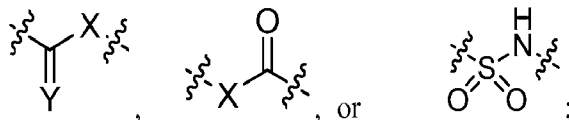


15 and pharmaceutically acceptable salts and prodrugs thereof.

A class of compounds described herein includes compounds having the following structure:

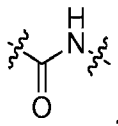


20 and pharmaceutically acceptable salts and prodrugs thereof. In these compounds, L is absent,



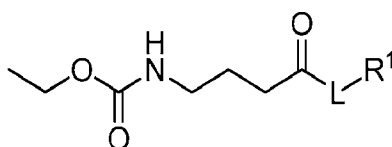
X is NR or O; Y is O or S; each R is independently selected from hydrogen or substituted or unsubstituted C₁₋₆ alkyl; and R¹ is hydrogen, alkoxy, substituted or unsubstituted amino, substituted or unsubstituted thiol, substituted or unsubstituted C₁₋₆ alkyl, substituted or unsubstituted C₂₋₆ alkenyl, substituted or unsubstituted aryl, substituted or unsubstituted

heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocyclyl. If L is absent, then R¹ is substituted or unsubstituted amino. Also, if L is



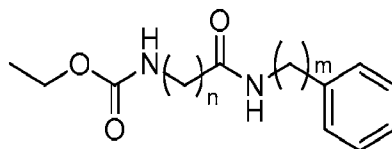
then R¹ is not ethoxyl.

A class of compounds described herein includes compounds having the following structure:



and pharmaceutically acceptable salts and prodrugs thereof. In these compounds, L is NH, O, S, or CH₂; and R¹ is substituted or unsubstituted C₁₋₆ alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. If L is NH, then R¹ is not -CH₂CH₂Ph.

A class of compounds described herein includes compounds having the following structure:



and pharmaceutically acceptable salts and prodrugs thereof. In these compounds, m is 0 to 5; and n is 1 to 5. If m is 2, then n is not 3. Optionally, m is 2.

Also provided herein are methods of inhibiting histone deacetylase (HDAC) activity in a cell. The methods of inhibiting HDAC activity in a cell involve contacting an HDAC isoform with a compound as described herein. Optionally, the HDAC isoform is HDAC-2. Optionally, the HDAC isoform is HDAC-6. The contacting can be performed *in vivo* or *in vitro*. Also provided herein are methods of inhibiting HDAC activity in a subject. The methods of inhibiting HDAC activity in a subject involve administering to the subject a compound as described herein. Optionally, the HDAC activity is HDAC-2 activity or HDAC-6 activity.

Further provided herein are methods of treating cancer and neurological disorders in a subject. The methods of treating cancer and neurological disorders in a subject involve administering to the subject an effective amount of a compound as described herein.

Optionally, the cancer is breast cancer, colon cancer, a hematological malignancy, or a

cutaneous T-cell lymphoma. The methods can further include administering a therapeutic agent (e.g., a chemotherapeutic agent, an anti-depressant, or an anxiolytic) to the subject.

Pharmaceutical formulations are further provided herein. The pharmaceutical formulations include a compound as described herein and a pharmaceutically acceptable carrier. Optionally, the pharmaceutical formulation is a solid pharmaceutical formulation. Optionally, the pharmaceutical formulation is a liquid pharmaceutical formulation and the pharmaceutically acceptable carrier is an aqueous medium.

The details of one or more embodiments are set forth in the description below. Other features, objects, and advantages will be apparent from the description and from the claims.

DETAILED DESCRIPTION

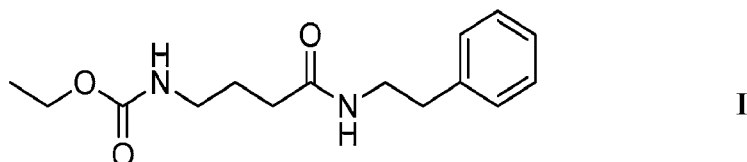
Bioactivity-guided fractionation of an extract derived from a dark brown cyanobacterium isolated during an expedition to the Coiba National Park on the Pacific coast of Panama led to the isolation of the new compound, designated Santacruzamate A (SCA).

Provided herein are compositions and analogs of Santacruzamate A. Also provided are methods for their use in inhibiting HDAC and in treating HDAC-related diseases (e.g., cancer and/or neurological disorders).

I. Compounds

a. Santacruzamate A

An HDAC inhibitor described herein includes Santacruzamate A, as represented by **Formula I**:

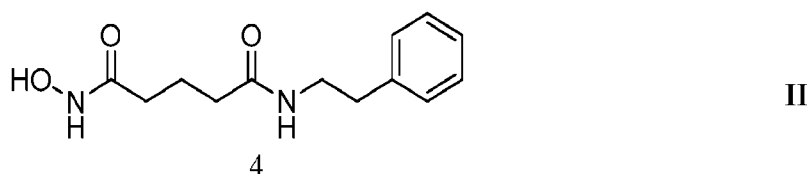


or a pharmaceutically acceptable salt or prodrug thereof.

Optionally, **Formula I** is synthetic Santacruzamate A (i.e., Santacruzamate A that is not obtained or extracted from a natural source).

b. Santacruzamate A Analogs

Further HDAC inhibitors described herein are Santacruzamate A analogs. A Santacruzamate A analog for use as an HDAC inhibitor described herein includes the compound represented by **Formula II**:

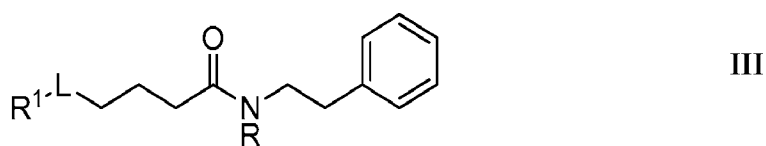


or a pharmaceutically acceptable salt or prodrug thereof.

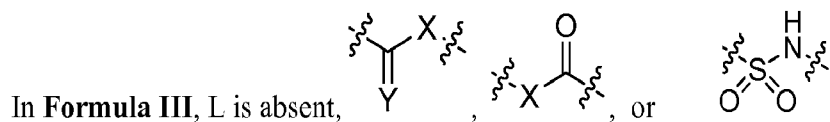
Santacruzamate A contains three structural motifs: a metal chelating moiety, which is the zinc binding group (ZBG); a surface recognition cap-group; and an aliphatic linker. Each of these structural motifs can be modified simultaneously (i.e., within the same molecule) to provide Santacruzamate A analogs. For purposes of demonstrating each of the possible modifications, **Formula III** (depicting modifications for the zinc binding group), **Formula IV** (depicting modifications for the cap-group), and **Formula V** (depicting modifications for the aliphatic linker group) are provided below. However, any of the modifications shown for any of **Formula III**, **Formula IV**, or **Formula V** can be combined with a separate modification shown for one of the other formulas. For example, **Formula III** modifications can be combined with **Formula IV** and/or **Formula V** modifications; **Formula IV** modifications can be combined with **Formula III** and/or **Formula V** modifications; and **Formula V** modifications can be combined with **Formula III** and/or **Formula IV** modifications.

i. Zinc Binding Group Analogs

The vast majority of existing HDAC inhibitors contain a hydroxamic acid zinc binding group. However, hydroxamic acids suffer from poor oral absorbance, rapid hydrolysis yielding poor pharmacokinetics, and strong non-specific affinity for metalloproteins. Described herein are HDAC inhibitors that are zinc binding group analogs of Santacruzamate A, as shown in **Formula III**.



or a pharmaceutically acceptable salt or prodrug thereof.



Also, in **Formula III**, X is NR or O.

Additionally, in **Formula III**, Y is O or S.

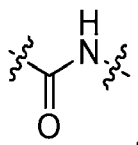
Further, in **Formula III**, each R is independently selected from hydrogen or substituted or unsubstituted C₁₋₆ alkyl. Optionally, each R is either hydrogen or methyl.

Also, in **Formula III**, R¹ is hydrogen, alkoxy, substituted or unsubstituted amino, substituted or unsubstituted thiol, substituted or unsubstituted C₁₋₆ alkyl, substituted or

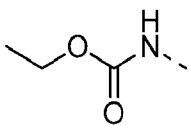
unsubstituted C₂₋₆ alkenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocyclyl.

In some examples of **Formula III**, if L is absent, then R¹ is substituted or
 5 unsubstituted amino.

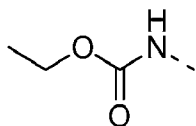
Also, in some examples of **Formula III**, if L is



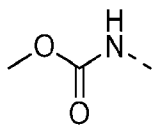
then R¹ is not ethoxyl, i.e., in some examples of **Formula III**, the L-R¹ group is not



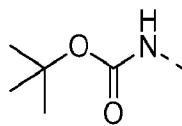
10 Examples of the L-R¹ group in **Formula III** include the following structures that form **Formula III** analogs:



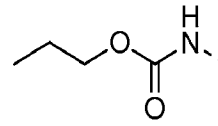
Analog III-1



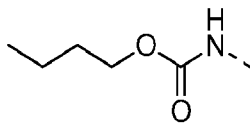
Analog III-2



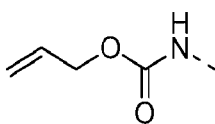
Analog III-3



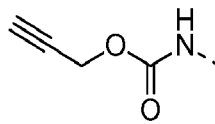
Analog III-4



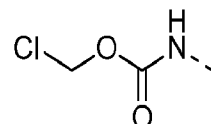
Analog III-5



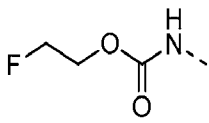
Analog III-6



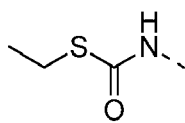
Analog III-7



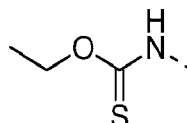
Analog III-8



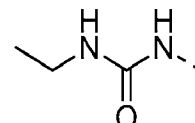
Analog III-9



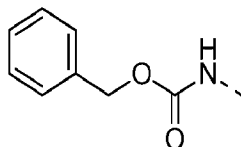
Analog III-10



Analog III-11



Analog III-12



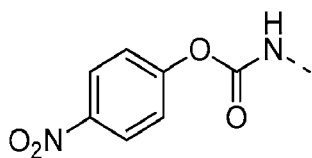
Analog III-13



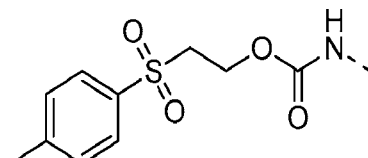
Analog III-14



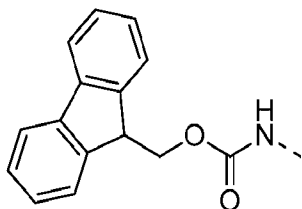
Analog III-15



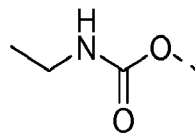
Analog III-16



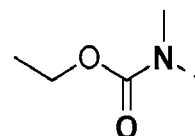
Analog III-17



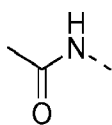
Analog III-18



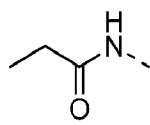
Analog III-21



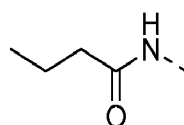
Analog III-22



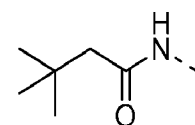
Analog III-23



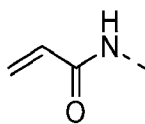
Analog III-24



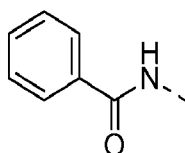
Analog III-25



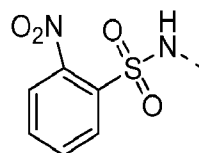
Analog III-26



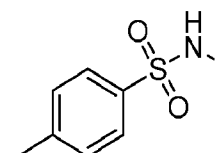
Analog III-27



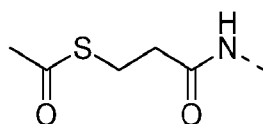
Analog III-28



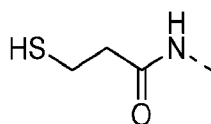
Analog III-29



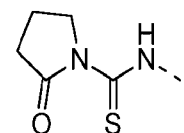
Analog III-30



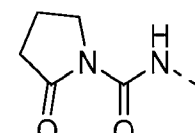
Analog III-32



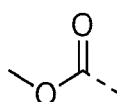
Analog III-33



Analog III-38



Analog III-39



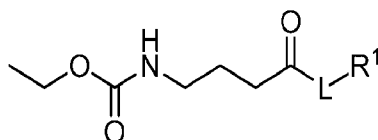
Analog III-40

In Analog III-22, each R group in **Formula III** is methyl.

ii. *Cap-Group Analogs*

Described herein are HDAC inhibitors that are cap-group analogs of Santacruzamate

5 A, as shown in **Formula IV**.



IV

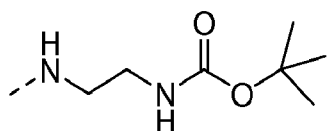
or a pharmaceutically acceptable salt or prodrug thereof.

In **Formula IV**, L is NH, O, S, or CH₂.

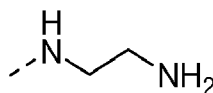
Also, in **Formula IV**, R¹ is substituted or unsubstituted C₁₋₆ alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

In **Formula IV**, if L is NH, then R¹ is not -CH₂CH₂Ph.

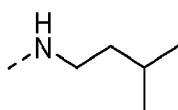
5 Examples of the L-R¹ group in **Formula IV** include the following structures that form **Formula IV** analogs:



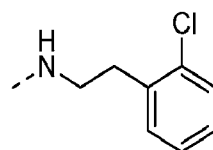
Analog IV-1



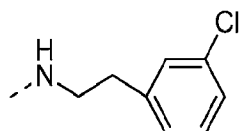
Analog IV-2



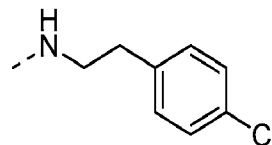
Analog IV-3



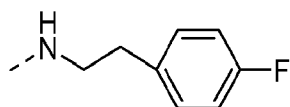
Analog IV-4a



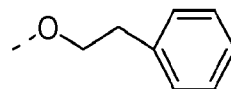
Analog IV-4b



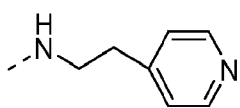
Analog IV-4c



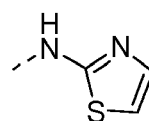
Analog IV-5



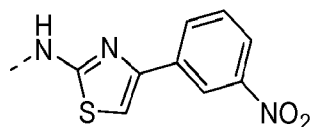
Analog IV-6



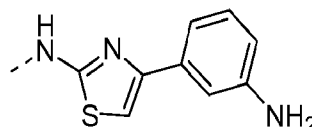
Analog IV-7



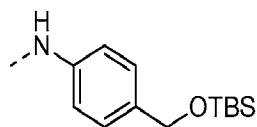
Analog IV-8



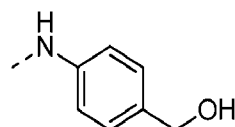
Analog IV-9



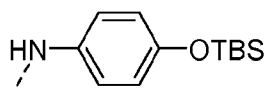
Analog IV-10



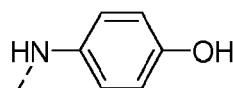
Analog IV-11



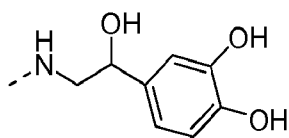
Analog IV-12



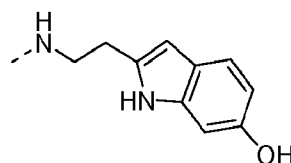
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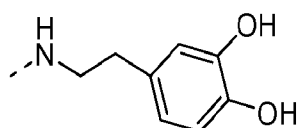
Analog IV-14



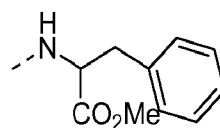
Analog IV-15



Analog IV-16



Analog IV-17

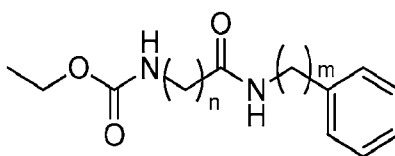


Analog IV-18

iii. Aliphatic Linker Group Analogs

Described herein are HDAC inhibitors that are aliphatic linker group analogs of Santacruzamate A, as shown in **Formula V**.

5



V

or a pharmaceutically acceptable salt or prodrug thereof.

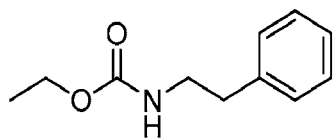
In **Formula V**, m is 0 to 5.

Also, in **Formula V**, n is 1 to 5.

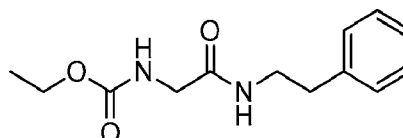
10

In **Formula V**, if m is 2, then n is not 3. Optionally, m is 2.

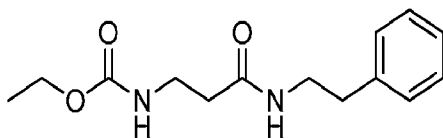
Examples of **Formula V** include the following compounds:



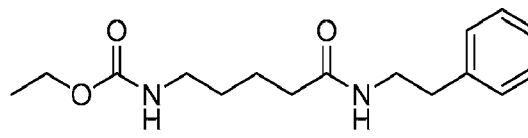
Analog V-19



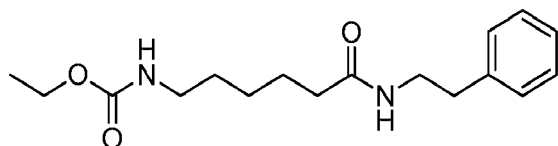
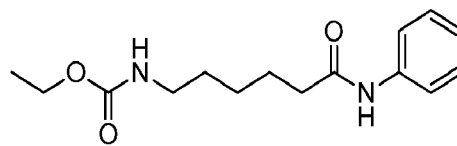
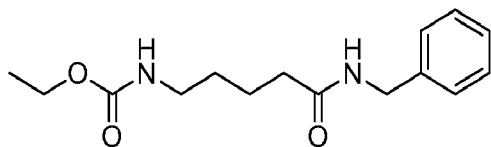
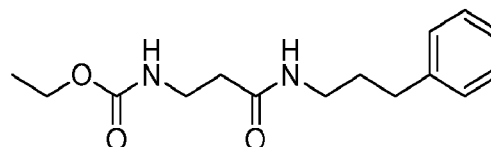
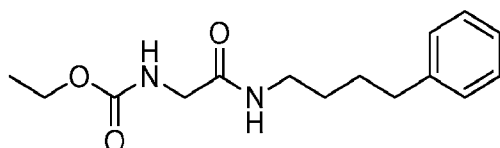
Analog V-20



Analog V-21



Analog V-22

**Analog V-23****Analog V-24****Analog V-25****Analog V-26****Analog V-27**

As used herein, the terms alkyl, alkenyl, and alkynyl include straight- and branched-chain monovalent substituents. Examples include methyl, ethyl, isobutyl, 3-butenyl, and the like. Ranges of these groups useful with the compounds and methods described herein include C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, and C₂-C₂₀ alkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₁-C₁₂ alkyl, C₂-C₁₂ alkenyl, C₂-C₁₂ alkynyl, C₁-C₆ alkyl, C₂-C₆ alkenyl, C₂-C₆ alkynyl, C₁-C₄ alkyl, C₂-C₄ alkenyl, and C₂-C₄ alkynyl.

Heteroalkyl, heteroalkenyl, and heteroalkynyl are defined similarly as alkyl, alkenyl, and alkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the backbone. Ranges of these groups useful with the compounds and methods described herein include C₁-C₂₀ heteroalkyl, C₂-C₂₀ heteroalkenyl, and C₂-C₂₀ heteroalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₁-C₁₂ heteroalkyl, C₂-C₁₂ heteroalkenyl, C₂-C₁₂ heteroalkynyl, C₁-C₆ heteroalkyl, C₂-C₆ heteroalkenyl, C₂-C₆ heteroalkynyl, C₁-C₄ heteroalkyl, C₂-C₄ heteroalkenyl, and C₂-C₄ heteroalkynyl.

The terms cycloalkyl, cycloalkenyl, and cycloalkynyl include cyclic alkyl groups having a single cyclic ring or multiple condensed rings. Examples include cyclohexyl, cyclopentylethyl, and adamantanyl. Ranges of these groups useful with the compounds and methods described herein include C₃-C₂₀ cycloalkyl, C₃-C₂₀ cycloalkenyl, and C₃-C₂₀

cycloalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₅-C₁₂ cycloalkyl, C₅-C₁₂ cycloalkenyl, C₅-C₁₂ cycloalkynyl, C₅-C₆ cycloalkyl, C₅-C₆ cycloalkenyl, and C₅-C₆ cycloalkynyl.

The terms heterocycloalkyl (i.e., heterocyclyl), heterocycloalkenyl, and
5 heterocycloalkynyl are defined similarly as cycloalkyl, cycloalkenyl, and cycloalkynyl, but can contain O, S, or N heteroatoms or combinations thereof within the cyclic backbone. Ranges of these groups useful with the compounds and methods described herein include C₃-C₂₀ heterocycloalkyl, C₃-C₂₀ heterocycloalkenyl, and C₃-C₂₀ heterocycloalkynyl. Additional ranges of these groups useful with the compounds and methods described herein include C₅-
10 C₁₂ heterocycloalkyl, C₅-C₁₂ heterocycloalkenyl, C₅-C₁₂ heterocycloalkynyl, C₅-C₆ heterocycloalkyl, C₅-C₆ heterocycloalkenyl, and C₅-C₆ heterocycloalkynyl.

Aryl molecules include, for example, cyclic hydrocarbons that incorporate one or more planar sets of, typically, six carbon atoms that are connected by delocalized electrons numbering the same as if they consisted of alternating single and double covalent bonds. An
15 example of an aryl molecule is benzene. Heteroaryl molecules include substitutions along their main cyclic chain of atoms such as O, N, or S. When heteroatoms are introduced, a set of five atoms, e.g., four carbon and a heteroatom, can create an aromatic system. Examples of heteroaryl molecules include furan, pyrrole, thiophene, imadazole, oxazole, pyridine, and pyrazine. Aryl and heteroaryl molecules can also include additional fused rings, for example,
20 benzofuran, indole, benzothiophene, naphthalene, anthracene, and quinoline. The aryl and heteroaryl molecules can be attached at any position on the ring, unless otherwise noted.

The alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycloalkyl, heterocycloalkenyl, or
heterocycloalkynyl molecules used herein can be substituted or unsubstituted. As used
25 herein, the term substituted includes the addition of an alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycloalkyl, heterocycloalkenyl, or heterocycloalkynyl group to a position attached to the main chain of the alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl, cycloalkenyl, cycloalkynyl, heterocycloalkyl, heterocycloalkenyl, or
30 heterocycloalkynyl, e.g., the replacement of a hydrogen by one of these molecules. Examples of substitution groups include, but are not limited to, hydroxyl, halogen (e.g., F, Br, Cl, or I), and carboxyl groups. Conversely, as used herein, the term unsubstituted indicates the alkyl, alkenyl, alkynyl, aryl, heteroalkyl, heteroalkenyl, heteroalkynyl, heteroaryl, cycloalkyl,

cycloalkenyl, cycloalkynyl, heterocycloalkyl, heterocycloalkenyl, or heterocycloalkynyl has a full complement of hydrogens, i.e., commensurate with its saturation level, with no substitutions, e.g., linear decane $-(CH_2)_9-CH_3$).

II. Pharmaceutical Formulations

5 The compounds described herein or derivatives thereof can be provided in a pharmaceutical composition. Depending on the intended mode of administration, the pharmaceutical composition can be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, or suspensions, preferably in unit dosage form suitable for single administration of a precise dosage. The
10 compositions will include a therapeutically effective amount of the compound described herein or derivatives thereof in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, or diluents. By pharmaceutically acceptable is meant a material that is not biologically or otherwise undesirable, which can be administered to an individual along with the selected compound
15 without causing unacceptable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained.

As used herein, the term carrier encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations. The choice of a carrier for use in a composition will depend
20 upon the intended route of administration for the composition. The preparation of pharmaceutically acceptable carriers and formulations containing these materials is described in, e.g., Remington's Pharmaceutical Sciences, 21st Edition, ed. University of the Sciences in Philadelphia, Lippincott, Williams & Wilkins, Philadelphia Pa., 2005. Examples of physiologically acceptable carriers include buffers, such as phosphate buffers, citrate buffer,
25 and buffers with other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates, including glucose, mannose, or dextrans; chelating agents, such as
30 EDTA; sugar alcohols, such as mannitol or sorbitol; salt-forming counterions, such as sodium; and/or nonionic surfactants, such as TWEEN[®] (ICI, Inc.; Bridgewater, New Jersey), polyethylene glycol (PEG), and PLURONICS[™] (BASF; Florham Park, NJ).

Compositions containing the compound described herein or derivatives thereof suitable for parenteral injection may involve physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

These compositions may also contain adjuvants, such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be promoted by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Isotonic agents, for example, sugars, sodium chloride, and the like may also be included. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Solid dosage forms for oral administration of the compounds described herein or derivatives thereof include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compounds described herein or derivatives thereof is admixed with at least one inert customary excipient (or carrier), such as sodium citrate or dicalcium phosphate, or (a) fillers or extenders, as for example, starches, lactose, sucrose, glucose, mannitol, and silicic acid, (b) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, (c) humectants, as for example, glycerol, (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate, (e) solution retarders, as for example, paraffin, (f) absorption accelerators, as for example, quaternary ammonium compounds, (g) wetting agents, as for example, cetyl alcohol, and glycerol monostearate, (h) adsorbents, as for example, kaolin and bentonite, and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, tablets, and pills, the dosage forms may also involve buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethyleneglycols, and the like.

5 Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others known in the art. They may contain opacifying agents and can also be of such composition that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions that can be used are polymeric substances and waxes. The active compounds can also be in micro-encapsulated form, if appropriate, with one or
10 more of the above-mentioned excipients.

Liquid dosage forms for oral administration of the compounds described herein or derivatives thereof include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents,
15 and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, and fatty acid esters of sorbitan, or mixtures of these substances, and the like.

20 Besides such inert diluents, the composition can also include additional agents, such as wetting, emulsifying, suspending, sweetening, flavoring, or perfuming agents.

Suspensions, in addition to the active compounds, may contain additional agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or
25 mixtures of these substances, and the like.

Compositions of the compounds described herein or derivatives thereof for rectal administrations are optionally suppositories, which can be prepared by mixing the compounds with suitable non-irritating excipients or carriers, such as cocoa butter, polyethyleneglycol or a suppository wax, which are solid at ordinary temperatures but liquid
30 at body temperature and, therefore, melt in the rectum or vaginal cavity and release the active component.

Dosage forms for topical administration of the compounds described herein or derivatives thereof include ointments, powders, sprays, and inhalants. The compounds

described herein or derivatives thereof are admixed under sterile conditions with a physiologically acceptable carrier and any preservatives, buffers, or propellants as may be required. Ophthalmic formulations, ointments, powders, and solutions are also contemplated as being within the scope of the compositions.

5 The compositions can include one or more of the compounds described herein and a pharmaceutically acceptable carrier. As used herein, the term pharmaceutically acceptable salt refers to those salts of the compound described herein or derivatives thereof that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of subjects without undue toxicity, irritation, allergic response, and the like, commensurate with
10 a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds described herein. The term salts refers to the relatively non-toxic, inorganic and organic acid addition salts of the compounds described herein. These salts can be prepared *in situ* during the isolation and purification of the compounds or by separately reacting the purified compound in its free base form with a
15 suitable organic or inorganic acid and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate, methane sulphonate, and laurylsulphonate salts, and the like. These may include cations
20 based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

25 Administration of the compounds and compositions described herein or pharmaceutically acceptable salts thereof can be carried out using therapeutically effective amounts of the compounds and compositions described herein or pharmaceutically acceptable salts thereof as described herein for periods of time effective to treat a disorder. The effective amount of the compounds and compositions described herein or pharmaceutically acceptable
30 salts thereof as described herein may be determined by one of ordinary skill in the art and includes exemplary dosage amounts for a mammal of from about 0.5 to about 200mg/kg of body weight of active compound per day, which may be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. Alternatively, the

dosage amount can be from about 0.5 to about 150mg/kg of body weight of active compound per day, about 0.5 to 100mg/kg of body weight of active compound per day, about 0.5 to about 75mg/kg of body weight of active compound per day, about 0.5 to about 50mg/kg of body weight of active compound per day, about 0.5 to about 25mg/kg of body weight of active compound per day, about 1 to about 20mg/kg of body weight of active compound per day, about 1 to about 10mg/kg of body weight of active compound per day, about 20mg/kg of body weight of active compound per day, about 10mg/kg of body weight of active compound per day, or about 5mg/kg of body weight of active compound per day. Those of skill in the art will understand that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors, including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition.

15 **III. Methods of Making the Compounds**

The compounds described herein can be prepared in a variety of ways known to one skilled in the art of organic synthesis or variations thereon as appreciated by those skilled in the art. The compounds described herein can be prepared from readily available starting materials. Optimum reaction conditions may vary with the particular reactants or solvents used, but such conditions can be determined by one skilled in the art.

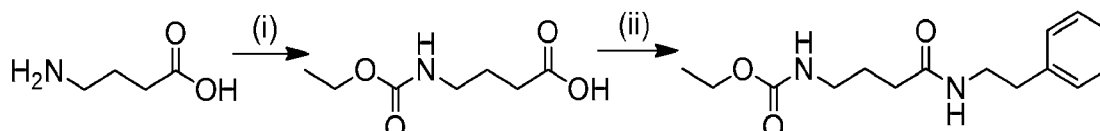
Variations on **Formula I**, **Formula II**, **Formula III**, **Formula IV**, and **Formula V** include the addition, subtraction, or movement of the various constituents as described for each compound. Similarly, when one or more chiral centers are present in a molecule, the chirality of the molecule can be changed. Additionally, compound synthesis can involve the protection and deprotection of various chemical groups. The use of protection and deprotection, and the selection of appropriate protecting groups can be determined by one skilled in the art.

Reactions to produce the compounds described herein can be carried out in solvents, which can be selected by one of skill in the art. Solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products under the conditions at which the reactions are carried out, i.e., temperature and pressure. Reactions can be carried out in one solvent or a mixture of more than one solvent. Product or intermediate formation can be monitored according to any suitable method known in the art. For example, product

formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (e.g., ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (e.g., UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

- 5 The compound described by **Formula I** can be made, for example, using reactions shown in **Scheme 1**.

Scheme 1:

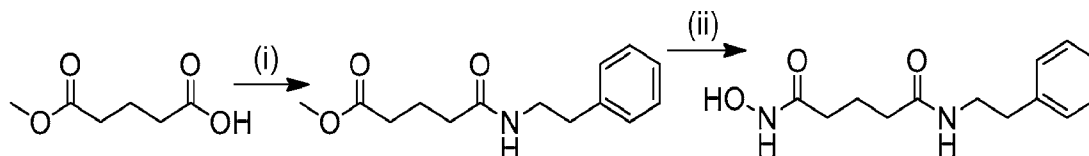


- 10 Reagents and conditions: (i) ethyl chloroformate, K_2CO_3 , THF, $0\text{ }^\circ\text{C}$ to rt, 76 % yield; (ii) phenethylamine, TEA, EDC-HCl, cat. DMAP, CH_2Cl_2 , $0\text{ }^\circ\text{C}$ to rt, 92 % yield.

A detailed synthetic procedure for preparing the compound described by **Formula I** is provided in Example 1 as Santacruzamate A (1).

- 15 The compound described by **Formula II** can be made, for example, using reactions shown in **Scheme 2**.

Scheme 2:

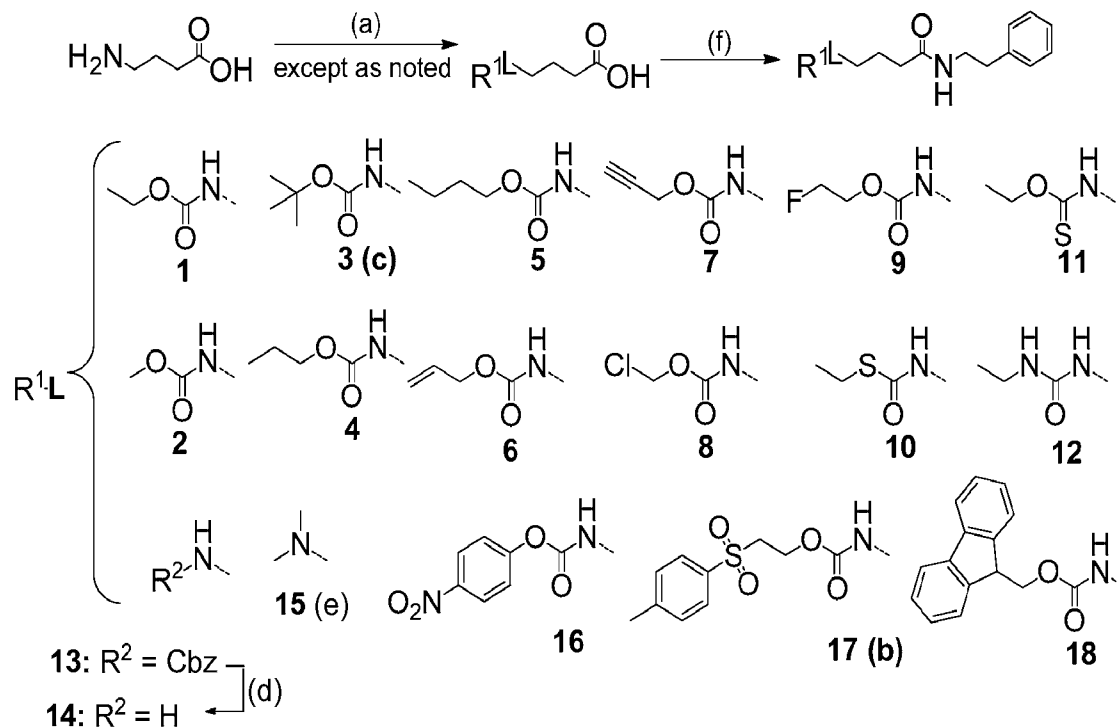


- 20 Reagents and conditions: (i) phenethylamine, TEA, EDC-HCl, cat. DMAP, CH_2Cl_2 , $0\text{ }^\circ\text{C}$ to rt, 88 % yield; (ii) hydroxylamine-HCl, KOH, rt, MeOH, 90 % yield.

A detailed synthetic procedure for preparing the compound described by **Formula II** is provided in Example 1 as SCA-SAHA hybrid (3).

- 25 Compounds described by **Formula III** can be made, for example, using reactions shown in **Schemes 3-8**. Compounds described by **Formula III** having L-R^1 groups as shown below can be prepared according to **Scheme 3**.

Scheme 3: Synthesis of Analogs III-1 through III-18

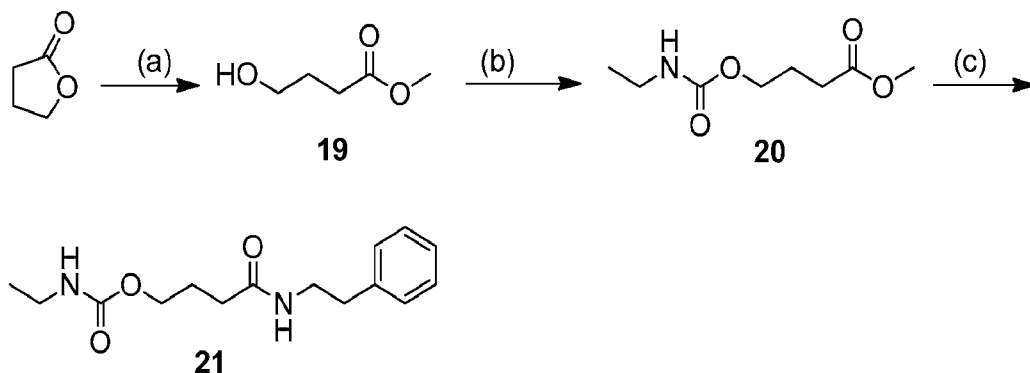


Reagent and conditions: (a) ClCOO-L-R¹, K₂CO₃ or NaOH, THF, 0 °C to room temp, 70-90%; (b) 2-*p*-Tolylsulphonyl ethyl chloroformate, MgO, THF, 0 °C to room temp, 52%; (c) Boc₂O, 1 M NaOH, THF, 0 °C to room temp, 97%; (d) H₂ (1 atm), Pd/C, MeOH, 99%; (e) Formalin, formic acid, reflux, 92% (f) Phenethylamine, EDC-HCl, TEA, cat. DMAP, CH₂Cl₂, 43 -93%.

Detailed synthetic procedures for preparing the compounds described by **Formula III** having the above-shown L-R¹ groups are provided in Example 2 as analogs III-1 through III-18.

A compound described by **Formula III** having an inverted ethyl carbamate L-R¹ groups can be prepared according to **Scheme 4**.

Scheme 4: Synthesis of Analog III-21

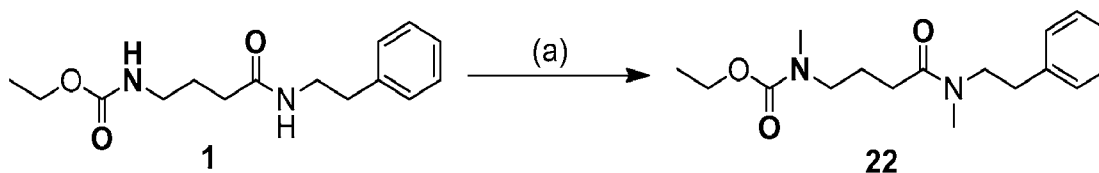


Reagents and Conditions: (a) TEA, MeOH, reflux: 92%; (b) (1) NHS, DCC, DMAP, DMF; 79% (2) 68% ethylamine, TEA, THF; 35%. (c) (1) LiOH, THF; (2) Phenethylamine, EDC-HCl, TEA, DMAP, CH₂Cl₂. 56% over two steps.

A detailed synthetic procedure for preparing the compound described by **Formula III** having an inverted ethyl carbamate L-R¹ group is provided in Example 2 as analog III-21.

A compound described by **Formula III** having N-methylated groups can be prepared according to **Scheme 5**.

Scheme 5: Synthesis of Analog III-22

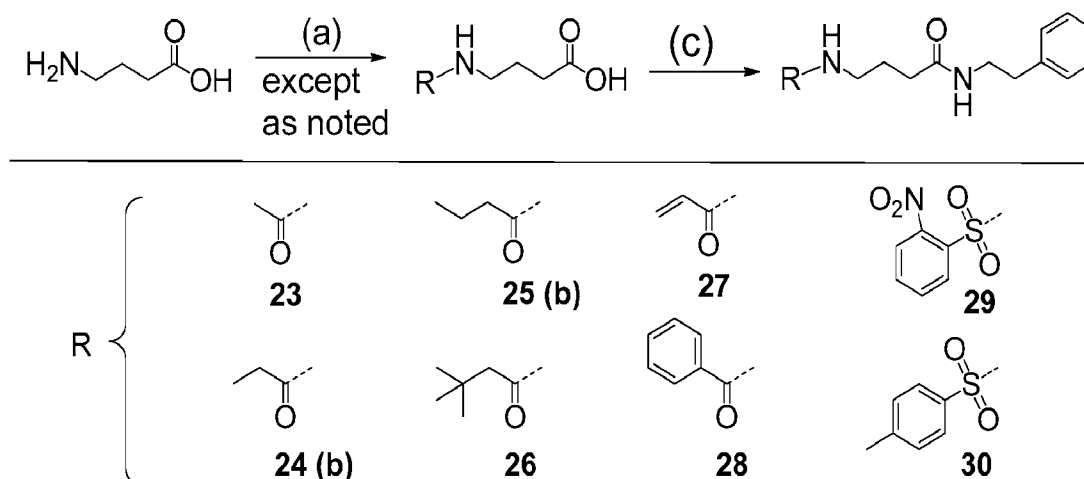


Reagents and Conditions: MeI, PPh₃, Hunig's base, CH₃CN, rt-48hrs 42%

A detailed synthetic procedure for preparing the compound described by **Formula III** having N-methylated groups is provided in Example 2 as analog III-22.

Compounds described by **Formula III** having N-amide L-R¹ groups can be prepared according to **Scheme 6**.

Scheme 6: Synthesis of Analog III-23 through III-30

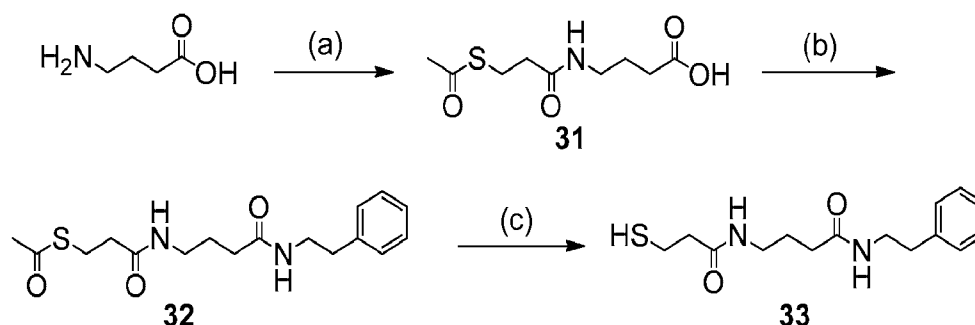


Reagents and conditions: (a) R-COCl , NaOH , $\text{THF/H}_2\text{O}$, 0°C to room temp (b) Propionic anhydride or butyric anhydride, cat H_2SO_4 , 100°C (c) Phenethylamine, EDC-HCl , TEA , cat. DMAP , CH_2Cl_2 .

Detailed synthetic procedures for preparing the compounds described by **Formula III** having N-amide L-R^1 groups are provided in Example 2 as analogs III-23 through III-30.

Compounds described by **Formula III** having terminal thiol containing L-R^1 groups can be prepared according to **Scheme 7**.

Scheme 7: Synthesis of Analogs III-32 and III-33

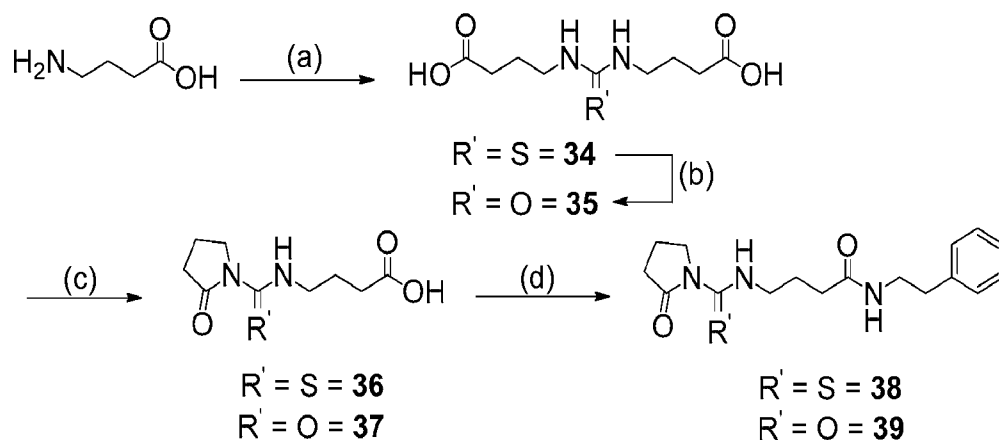


Reagents and conditions: (a) $\text{S-2-(chlorocarbonyl)ethyl ethanethioate}$, Na_2CO_3 , $\text{EtOAc/H}_2\text{O}$, 0°C - RT (b) Phenethylamine, EDC-HCl , TEA , cat. DMAP , CH_2Cl_2 (c) Acetyl chloride, MeOH , 6 hours, room temperature.

Detailed synthetic procedures for preparing the compounds described by **Formula III** having the terminal thiol L-R^1 groups are provided in Example 2 as analogs III-32 and III-33.

Compounds described by **Formula III** having pyrrolidone containing L-R^1 groups can be prepared according to **Scheme 8**.

Scheme 8: Synthesis of Analogs III-38 and III-39

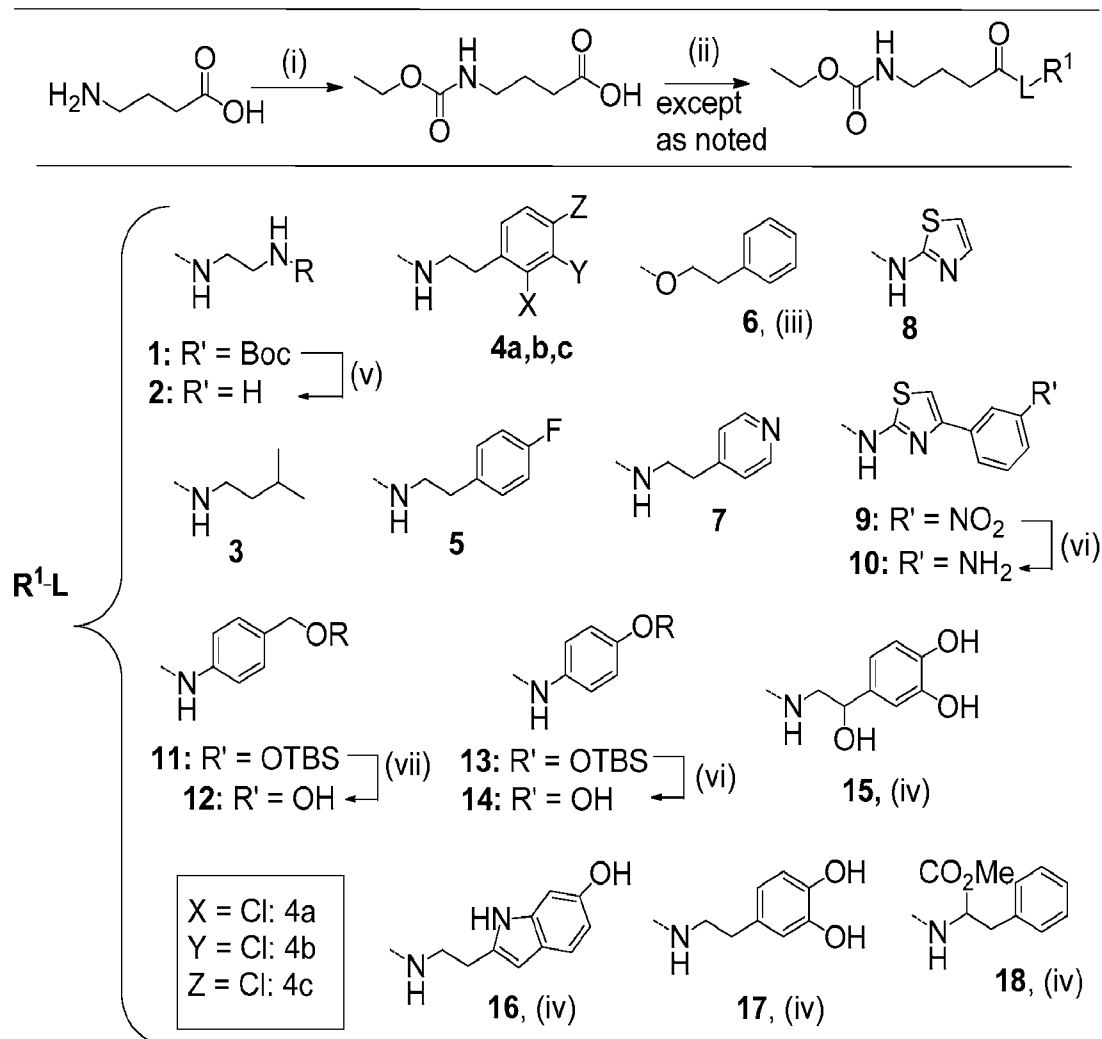


5 Reagents and conditions: (a) (1) NaOH, CS₂, 40 °C (2) reflux, 7 h, 98% (b) H₂O₂, KOH, H₂O
 (c) cat. p-TsOH, neat 185 °C, 30 torr, 15-20 min (d) Phenethylamine, EDC-HCl, TEA, cat.
 DMAP, CH₂Cl₂.

Detailed synthetic procedures for preparing the compounds described by **Formula III**
 having pyrrolidone L-R¹ groups are provided in Example 2 as analogs III-38 and III-39.

10 Compounds described by **Formula IV** can be made, for example, using reactions
 shown in **Scheme 9**.

Scheme 9: Synthesis of Analogs IV-1 through IV-18

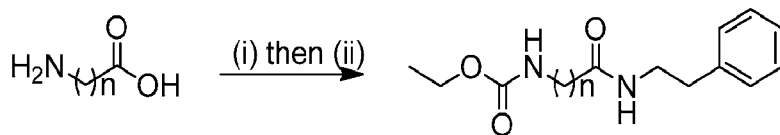


Reagent and conditions: (i) Ethyl chloroformate, K_2CO_3 , H_2O , 0°C to room temperature, 48 hours; (ii) cap-group L-R^1 , EDC-HCl, TEA, catalytic DMAP, CH_2Cl_2 ; (iii) EDC-HCl, DIPEA, HOBT, DMF; (iv) PyBOP, HOBT, DIPEA, ACN; Compounds 15-18 installed as O-TMS and deprotected in situ via 2M HCl workup; (v) TFA: H_2O (10:1) 45 minutes, room temperature; (vi) H_2 , Pd-C, EtOH, AcOH. (vii) THF, TBAF, 24 hours, rt

Detailed synthetic procedures for preparing the compounds described by **Formula IV** having the above-shown L-R^1 groups are provided in Example 3 as analogs IV-1 through IV-18.

Compounds described by **Formula V** where m is 2 and n is 0, 1, 2, 4, or 5 can be prepared according to **Scheme 10**.

Scheme 10: Synthesis of Analogs V-19 through V-23



19: n=0

20: n=1

21: n=2

22: n=4

23: n=5

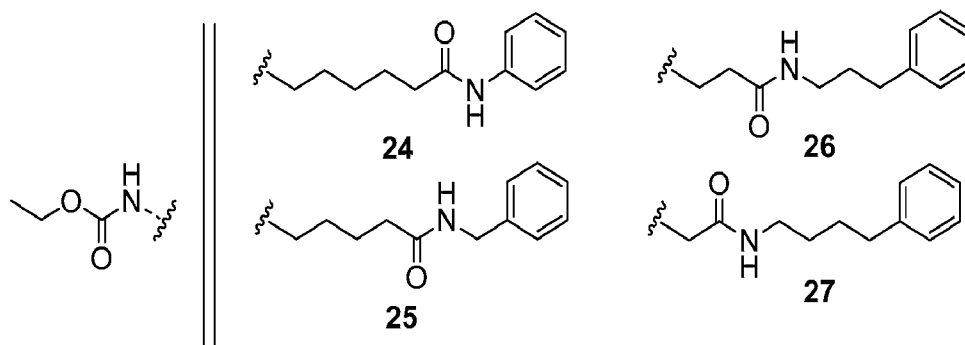
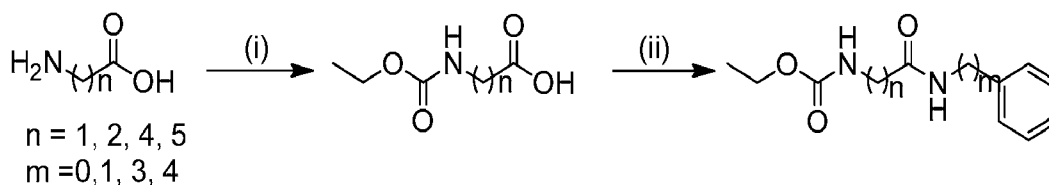
Reagent and conditions: (i) Ethyl chloroformate, K_2CO_3 , H_2O , $0^\circ C$ to room temp, ;
 (ii) Phenethylamine, EDC-HCl, TEA, cat. DMAP, CH_2Cl_2 .

Detailed synthetic procedures for preparing the compounds described by **Formula V** where m is 2 and n is 0, 1, 2, 4, or 5 are provided in Example 3 as analogs V-19 through V-

5 23.

Compounds described by **Formula V** where m is 0, 1, 3, or 4 and n is 0, 1, 2, 4, or 5 can be prepared according to **Scheme 11**.

Scheme 11: Synthesis of Analogs V-24 through V-27



Reagent and conditions: (i) Ethyl chloroformate, K_2CO_3 , H_2O , $0^\circ C$ to room temp; (ii)
 Phenethylamine, EDC-HCl, TEA, cat. DMAP, CH_2Cl_2 .

10

Detailed synthetic procedures for preparing the compounds described by **Formula V** where m is 0, 1, 3, or 4 and n is 1, 2, 4, or 5 are provided in Example 3 as analogs V-24 through V-27.

15

IV. Methods of Use

Provided herein are methods to treat, prevent, or ameliorate HDAC-related diseases in a subject. Optionally, the HDAC-related disease is cancer, a neurological disorder, or asthma. The methods include administering to a subject an effective amount of one or more of the
5 compounds or compositions described herein, or a pharmaceutically acceptable salt or prodrug thereof. The expression “effective amount,” when used to describe an amount of compound in a method, refers to the amount of a compound that achieves the desired pharmacological effect or other effect, for example, an amount that results in tumor growth rate reduction. The compounds and compositions described herein or pharmaceutically
10 acceptable salts thereof are useful for treating cancer and/or neurological disorders in humans, including, without limitation, pediatric and geriatric populations, and in animals, e.g., veterinary applications.

Optionally, the cancer is breast cancer, colon cancer, a hematological malignancy, or a cutaneous T-cell lymphoma. Optionally, the cancer is bladder cancer, brain cancer,
15 colorectal cancer, cervical cancer, gastrointestinal cancer, genitourinary cancer, head and neck cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, or testicular cancer.

Optionally, the neurological disorder involves brain damage, a brain dysfunction, a spinal cord disorder, a peripheral nervous system disorder (e.g., peripheral neuropathy), a
20 cranial nerve disorder, an autonomic nervous system disorder, a seizure disorder, a movement disorder, a sleep disorder, a metabolic disorder, a migraine, back pain, neck pain, a central neuropathy, or a neuropsychiatric illness. Optionally, the neurological disorder is a neurodegenerative disorder, such as, for example, Alexander disease, Alper’s disease, Alzheimer disease, amyotrophic lateral sclerosis, ataxia telangiectasia, Batten disease (also
25 known as Spielmeyer-Vogt-Sjogren-Batten disease), Canavan disease, Cockayne syndrome, Corticobasal degeneration, Creutzfeldt-Jakob disease, Huntington disease, Kennedy’s disease, Krabbe disease, Lewy body dementia, Machado-Joseph disease, Spinocerebellar ataxia type 3, multiple sclerosis, multiple system atrophy, Parkinson’s disease, Pelizaeus-Merzbacher disease, Pick’s disease, Primary lateral sclerosis, Refsum’s disease, Sandhoff
30 disease, Schilder’s disease, Spielmeyer-Vogt-Sjogren-Batten disease (also known as Batten disease), Spinocerebellar ataxia (multiple types with varying characteristics), Spinal muscular atrophy, Steele-Richardson-Olszewski disease, Tay-Sachs, Transmissible spongiform encephalopathies (TSE), and Tabes dorsalis.

The method of treating or preventing cancer and/or neurological disorders in a subject can further involve administering to the subject a therapeutic agent. Thus, the provided compositions and methods can include one or more additional agents. The one or more additional agents and the compounds described herein or pharmaceutically acceptable salts or prodrugs thereof can be administered in any order, including concomitant, simultaneous, or sequential administration. Sequential administration can be temporally spaced on the order of up to several days apart. The methods can also include more than a single administration of the one or more additional agents and/or the compounds described herein or pharmaceutically acceptable salts or prodrugs thereof. The administration of the one or more additional agents and the compounds described herein or pharmaceutically acceptable salts or prodrugs thereof can be by the same or different routes and concurrently or sequentially.

Therapeutic agents include, but are not limited to, chemotherapeutic agents. A chemotherapeutic agent is a compound or composition effective in inhibiting or arresting the growth of an abnormally growing cell. Thus, such an agent may be used therapeutically to treat cancer as well as other diseases marked by abnormal cell growth. Illustrative examples of chemotherapeutic compounds include, but are not limited to, bexarotene, gefitinib, erlotinib, gemcitabine, paclitaxel, docetaxel, topotecan, irinotecan, temozolomide, carmustine, vinorelbine, capecitabine, leucovorin, oxaliplatin, bevacizumab, cetuximab, panitumumab, bortezomib, oblimersen, hexamethylmelamine, ifosfamide, CPT-11, deflunomide, cycloheximide, dicarbazine, asparaginase, mitotant, vinblastine sulfate, carboplatin, colchicine, etoposide, melphalan, 6-mercaptopurine, teniposide, vinblastine, antibiotic derivatives (e.g. anthracyclines such as doxorubicin, liposomal doxorubicin, and diethylstilbestrol doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil (FU), 5-FU, methotrexate, floxuridine, interferon alpha-2B, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cisplatin, vincristine and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chlorambucil, mechlorethamine (nitrogen mustard) and thiotepa); and steroids (e.g., bethamethasone sodium phosphate).

- Optionally, the therapeutic agent can be an anti-depressant. Illustrative examples of anti-depressant compounds include, but are not limited to, adatsanserin hydrochloride; adinazolam; adinazolam mesylate; alaproclate; aletamine hydrochloride; amedalin hydrochloride; amitriptyline hydrochloride; amoxapine; aptazapine maleate; azaloxan fumarate; azepindole; azipramine hydrochloride; bipenamol hydrochloride; bupropion hydrochloride; butacetin; butriptyline hydrochloride; caroxazone; cartazolate; ciclazindol; cidoxepin hydrochloride; cilobamine mesylate; clodazon hydrochloride; clomipramine hydrochloride; cotinine fumarate; cyclindole; cypenamine hydrochloride; cyprolidol hydrochloride; cyproximide; daledalin tosylate; dapoxetine hydrochloride; dazadrol maleate; dazepinil hydrochloride; desipramine hydrochloride; dexamisole; deximafen; dibenzepin hydrochloride; dioxadrol hydrochloride; dothiepin hydrochloride; doxepin hydrochloride; duloxetine hydrochloride; eclanamine maleate; encyprate; etoperidone hydrochloride; fantridone hydrochloride; fehmetozole hydrochloride; fenmetramide; fezolamine fumarate; fluotracen hydrochloride; fluoxetine; fluoxetine hydrochloride; fluparoxan hydrochloride; gamfexine; guanoxyfen sulfate; imafen hydrochloride; imiloxan hydrochloride; imipramine hydrochloride; indeloxazine hydrochloride; intriptyline hydrochloride; iprindole; isocarboxazid; ketipramine fumarate; lofepramine hydrochloride; lortalamine; maprotiline; maprotiline hydrochloride; melitracen hydrochloride; milacemide hydrochloride; minaprine hydrochloride; mirtazapine; moclobemide; modaline sulfate; napactadine hydrochloride; napamezole hydrochloride; nefazodone hydrochloride; nisooxetine; nitrafudam hydrochloride; nomifensine maleate; nortriptyline hydrochloride; octriptyline phosphate; opipramol hydrochloride; oxaprotiline hydrochloride; oxypertine; paroxetine; phenelzine sulfate; pirandamine hydrochloride; pizotyline; pridefine hydrochloride; prolintane hydrochloride; protriptyline hydrochloride; quipazine maleate; rolicyprine; seproxetine hydrochloride; sertraline hydrochloride; sibutramine hydrochloride; sulpiride; suritozole; tametraline hydrochloride; tampramine fumarate; tandamine hydrochloride; thiazesim hydrochloride; thozalinone; tomoxetine hydrochloride; trazodone hydrochloride; trebenzomine hydrochloride; trimipramine; trimipramine maleate; venlafaxine hydrochloride; viloxazine hydrochloride; zimeldine hydrochloride; and zometapine.
- Further, the therapeutic agent can be an anxiolytic. Illustrative examples of anxiolytic compounds include, but are not limited to, alprazolam; chlordiazepoxide; clonazepam; diazepam; lorazepam; tofisopam; buspirone; tandospirone; gepirone; barbiturates; hydroxyzine; pregbalin; chlorpheniramine; and diphenhydramine.

Any of the aforementioned therapeutic agents can be used in any combination with the compositions described herein. Combinations are administered either concomitantly (e.g., as an admixture), separately but simultaneously (e.g., via separate intravenous lines into the same subject), or sequentially (e.g., one of the compounds or agents is given first followed by the second). Thus, the term combination is used to refer to concomitant, simultaneous, or sequential administration of two or more agents.

The methods and compounds as described herein are useful for both prophylactic and therapeutic treatment. For prophylactic use, a therapeutically effective amount of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein are administered to a subject prior to onset (e.g., before obvious signs of cancer or a neurologic disorder), during early onset (e.g., upon initial signs and symptoms of cancer or a neurologic disorder), or after the development of cancer or a neurologic disorder.

Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of cancer or a neurologic disorder. Therapeutic treatment involves administering to a subject a therapeutically effective amount of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein after cancer or a neurologic disorder is diagnosed.

The methods and compounds described herein are also useful in inhibiting HDAC activity in a cell or in a subject. The methods of inhibiting HDAC activity in a cell include contacting an HDAC isoform with an effective amount of one or more compounds as described herein. The HDAC isoform can be, for example, a Class I HDAC isoform or a Class II HDAC isoform. Optionally, the HDAC isoform can be HDAC-2 or HDAC-6. The contacting can be performed *in vivo* or *in vitro*. The methods of inhibiting HDAC activity in a subject include administering to an effective amount of one or more compounds as described herein. Optionally, the HDAC activity is HDAC-2 activity or HDAC-6 activity.

V. Assays

The enzymatic activity of the compounds provided herein as inhibitors of HDAC may be measured in standard assays, e.g., enzymatic or cellular assays. Compounds that are identified as HDAC inhibitors are useful in treating or preventing HDAC-related diseases (e.g., cancer and/or neurologic disorders). The activities of the compounds as determined using the assays described herein can be reported in terms of IC_{50} . As used herein, IC_{50} refers to an amount, concentration, or dosage of a particular test compound that achieves a 50%

inhibition of a maximal response in an assay that measures such response. Suitable assays are described in Examples 1-3.

VI. Kits

Also provided herein are kits for treating or preventing HDAC-related diseases (e.g., cancer and/or a neurologic disorder) in a subject. A kit can include any of the compounds or compositions described herein. For example, a kit can include a compound of **Formula I**, **Formula II**, **Formula III**, **Formula IV**, **Formula V**, or combinations thereof. A kit can further include one or more additional agents, such as a chemotherapeutic agent (e.g., gemcitabine, paclitaxel, or tamoxifen), an anti-depressant (e.g., amitriptyline, duloxetine, or sertraline), and/or an anxiolytic (e.g., benzodiazepines, azapirones, or diphenhydramine). A kit can include an oral formulation of any of the compounds or compositions described herein. A kit can additionally include directions for use of the kit (e.g., instructions for treating a subject), a container, a means for administering the compounds or compositions, and/or a carrier.

As used herein the terms treatment, treat, or treating refer to a method of reducing one or more symptoms of a disease or condition. Thus in the disclosed method, treatment can refer to a reduction by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% in the severity of one or more symptoms of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms or signs (e.g., size of the tumor or rate of tumor growth) of the disease in a subject as compared to a control. As used herein, control refers to the untreated condition (e.g., the tumor cells not treated with the compounds and compositions described herein). Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

As used herein, the terms prevent, preventing, and prevention of a disease or disorder refer to an action, for example, administration of a composition or therapeutic agent, that occurs before or at about the same time a subject begins to show one or more symptoms of the disease or disorder, which inhibits or delays onset or severity of one or more symptoms of the disease or disorder.

As used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater, or any percent change in between 10% and greater than 90%, as compared to a control level. Such terms can include, but do not necessarily include, complete elimination.

5 As used herein, subject means both mammals and non-mammals. Mammals include, for example, humans; non-human primates, e.g., apes and monkeys; cattle; horses; sheep; rats; mice; pigs; and goats. Non-mammals include, for example, fish and birds.

The examples below are intended to further illustrate certain aspects of the methods and compositions described herein, and are not intended to limit the scope of the claims.

10

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary. Efforts have been made to ensure accuracy with respect to numbers (e.g.,
15 amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C. or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be
20 used to optimize the product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

The following abbreviations used herein as defined as follows: “calcd” refers to calculated; “cat.” refers to a catalytic amount; “conc” refers to concentrated; “h”, “hr”, or “hrs” refers to hour or hours; “min” refers to minute or minutes; “mp” refers to melting point;
25 “obs.” refers to observed; “rt” refers to room temperature; “sec” refers to second or seconds; and “sat” refers to saturated.

Example 1: Characterization and Synthesis of Santacruzamate A

Described herein is the isolation of an isozyme selective HDAC inhibitor with unprecedented potency against Class I HDAC enzymes. Due to the collection of the source
30 organism from near Santa Cruz Island in Panama’s Coiba National Park, a UNESCO World Heritage Site, this carbamate derivative has been named Santacruzamate A (SCA).

Experimental:

General Experimental Procedures. IR spectra were recorded on a Shimadzu FT-IR 8400 spectrometer. Melting points were collected on a Mel-Temp digital melting point apparatus. NMR spectra for the natural product SCA (**1**) were obtained on a JEOL Eclipse 400 MHz spectrometer and referenced to TMS. All other NMR spectra were recorded on a
5 Brüker Avance 500 (500.13 MHz ^1H , 125.65 MHz ^{13}C) with chemical shifts given in ppm downfield from TMS. LC-MS data were collected on an Agilent ESI single quadrupole mass spectrometer coupled to an Agilent HPLC system with a G1311 quaternary pump, G1322 degasser, and a G1315 diode array detector using an Eclipse XDB-C₁₈ (4.6 x 150 mm, 5 μm) RP-HPLC column. High resolution mass spectrometric data (HRMS) were collected on a
10 Micromass VB-QTOF tandem mass spectrometer. HPLC purifications of natural product isolates were carried out on a Merck Hitachi LaChrom HPLC system with a L-7100 pump, L-7614 degasser, and L-7455 diode array detector using a Prontosil-120 C₁₈ (4.6 x 250 mm, 5 μm) RP-HPLC column, with solvent systems as indicated below. All chemicals were used as received from Sigma-Aldrich or Acros without further purification. Hexanes,
15 tetrahydrofuran (THF), diethyl ether (Et₂O), and dichloromethane (CH₂Cl₂) for synthesis were used directly from a Baker Cycle-tainer system. All glassware was flame-dried under vacuum, and all reactions were performed under an argon atmosphere, unless otherwise noted. Thin-layer chromatography (TLC) was done on Fluka glass-backed TLC plates with fluorescent indicator and 0.2 mm silica gel layer thickness, and *p*-anisaldehyde was used as a
20 developing agent. Column chromatography was done using 60 Å porosity, 32-63 μm silica gel. Structural integrity and purity of the test compounds were determined by the composite of ^1H and ^{13}C NMR, melting point range, LC-MS and HRMS and compounds were found to be > 95% pure.

Collection and Extraction. A cyanobacterium morphologically resembling the genus
25 *Symploca* was collected in March 2007 by hand using SCUBA at depths of 30-45 feet. The collection site was a coral and rock reef in the Coiba National Park (7° 37.980 N, 81° 47.091 W) in Veraguas, Panama. After straining through a mesh bag to remove excess seawater, the sample was stored in 1:1 EtOH:sea water at -20 °C. The voucher specimen, number PAC-03/03/2007-1, is deposited at Scripps Institution of Oceanography, UCSD, San Diego, CA.
30 The sample (221.5 g dry weight) was thawed and exhaustively extracted with 2:1 CH₂Cl₂/MeOH. After solvent evaporation, 2.1 g of crude organic extract was obtained. The extract was fractionated using flash Si gel column chromatography (Aldrich, Si gel 60, 230-400 mesh, 40 x 180 mm) using 300 mL each of 100% hexanes (A), 9:1 hexanes/EtOAc (B),

4:1 hexanes/EtOAc (C), 3:2 hexanes/EtOAc (D), 2:3 hexanes/EtOAc (E), 1:4 hexanes/EtOAc (F), 100% EtOAc (G), 3:1 EtOAc/MeOH (H), and 100% MeOH (I). Fraction H exhibited strong anti-malarial activity (99.9% inhibition of parasite growth at 10 µg/mL) and strong anti-cancer activity (50% MCF-7 breast cancer cell death at 10 µg/mL) and was thus
 5 subjected to further fractionation using a Burdick & Jackson C₁₈ RP-SPE cartridge with a MeOH-H₂O solvent gradient (1:1, 3:2, 7:3, 4:1 MeOH/EtOAc, 100% MeOH, 100% EtOAc). The fraction eluting with 1:1 MeOH:H₂O was subjected to RP-HPLC purification (55% MeOH/45% H₂O, 1.0 mL/min) to yield santacruzamate A (SCA, **1**, 4.0 mg, *t_R* 10.9 min, 0.19% of extract).

10 *Santacruzamate A (SCA, 1)*: white amorphous solid; mp 112-113 °C; IR $\nu_{\max}$ (film) 3345, 3288, 1703, 1699, 1655, 1543, 1538, 1446, 1307, 1285, 1249, 1223, 1139, 1050, 1031; ¹H and ¹³C NMR data (CDCl₃, 400 and 100 MHz, respectively), see Table 1; ESI-MS *m/z* (%) 301.1 (8, [M+Na]⁺), 280.2 (25), 279.3 (100, [M+H]⁺); HRESI-MS [M+H]⁺ *m/z* 279.1721 (calculated for C₁₅H₂₃N₂O₃, 279.1709).

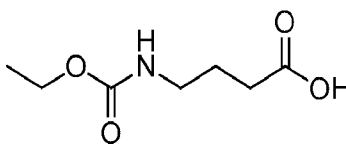
15 *Morphological Characterization*. Morphological characterization was performed using an Olympus IX51 epifluorescent microscope (1000X) equipped with an Olympus U-CMAD3 camera. Measurements were provided as: mean ± standard deviation (SD). The filament means were the average of three filament measurements, and cell measurements the average of ten adjacent cells in each of three filaments. Morphological comparison and
 20 putative taxonomic identification of the cyanobacterial specimen was performed in accordance with modern classification systems.

Gene Sequencing. Cyanobacterial specimens were preserved for genetic analysis both as live material and in 10 mL RNAlater (Ambion). Algal biomass (~50 mg) was partly cleaned under an Olympus VMZ dissecting microscope. Genomic DNA was extracted using
 25 the WIZARD Genomic DNA Purification Kit (Promega) following the manufacturer's specifications. DNA concentration and purity was measured on a DU® 800 spectrophotometer (Beckman Coulter). The 16S rRNA genes were PCR-amplified from isolated DNA using the general primer set 106F and 1509R while subsequent PCR reactions were performed using the modified lineage-specific primers. The PCR reaction volumes
 30 were 25 µL containing 0.5 µL (~50 ng) of DNA, 2.5 µL of 10 × PfuUltra IV reaction buffer, 0.5 µL (25 mM) of dNTP mix, 0.5 µL of each primer (10 µM), 0.5 µL of PfuUltra IV fusion HS DNA polymerase, and 20.5 µL dH₂O. The PCR reactions were performed with an Eppendorf® Mastercycler® gradient as follows: initial denaturation for 2 minutes at 95 °C, 25

cycles of amplification, followed by 20 seconds at 95 °C, 20 seconds at 55 °C and 1.5 minutes at 72 °C, and final elongation for 3 minutes at 72 °C. PCR products were analyzed on a (1%) agarose-gel in SB buffer and visualized by EtBr staining. The PCR products were purified using a MinElute[®] PCR Purification Kit (Qiagen) before subcloning using the Zero Blunt[®] TOPO[®] PCR Cloning Kit (Invitrogen) following the manufacturer's specifications. Plasmid DNA was isolated using the QIAprep[®] Spin Miniprep Kit (Qiagen) and sequenced with M13 primers. The 16S rRNA gene sequence is available in the DDBJ/EMBL/GenBank databases under accession number JX458089-1.

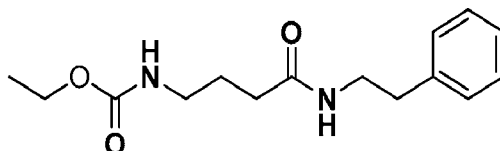
Phylogenetic Inference. The 16S rRNA gene sequence of PAC-19-FEB-10-1 was aligned with evolutionarily informative cyanobacteria using the L-INS-I algorithm in MAFFT 6.717 and refined using the SSU secondary structures model for *Escherichia coli* J01695 without data exclusion. The best-fitting nucleotide substitution model optimized by maximum likelihood (ML) was selected using corrected Akaike/Bayesian Information Criterion (AIC_C/BIC) in jModeltest 0.1.1. The evolutionary histories of the cyanobacterial genes were inferred using ML and Bayesian inference (BI) algorithms. The ML inference was performed using PhyML for the GTR+I+G model assuming heterogeneous substitution rates and gamma substitution of variable sites (proportion of invariable sites (pINV) = 0.495, shape parameter (α) = 0.452, number of rate categories = 4) with 1,000 bootstrap-replicates. The Bayesian inference was conducted using MrBayes 3.1 with four Metropolis-coupled MCMC chains (one cold and three heated) run for 1,000,000 generations. The first 25% were discarded as burn-in and the following data set was sampled with a frequency of every 100 generations.

Synthesis of SCA (1) and SCA-SAHA hybrid (3). Full experimental details are provided below for synthetic intermediates and products of SCA (**1** or **Formula I**) and the SCA-SAHA hybrid (**3** or **Formula II**).



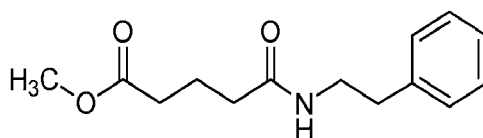
4-((ethoxycarbonyl)amino)butanoic acid: γ -Aminobutyric acid (0.50 g, 4.85 mmol) was dissolved in H₂O (7 mL). Once dissolved, K₂CO₃ (1.74 g, 12.6 mmol) was added and the resulting solution was cooled to 0 °C. Ethyl chloroformate (0.63 mL, 6.31 mmol) was added dropwise and the solution was stirred at 0 °C for 2 hours and then stirred overnight at room temperature. The reaction mixture was then diluted with H₂O (20 mL) and extracted

with EtOAc (3 x 10 mL). The aqueous phase was acidified to pH 2 with cold concentrated HCl and extracted with EtOAc (3 x 20 mL). The EtOAc partitions were dried over Na₂SO₄ and concentrated to reveal a white solid, which was recrystallized from cold hexanes to yield long white crystals (0.645 g, 3.68 mmol, 76% yield): melting point (mp) 44.1-45.0 °C; ¹H NMR (500 MHz, methanol-*d*₄) δ 4.12 (q, *J* = 6.94 Hz, 2H), 2.85 (t, *J* = 6.94 Hz, 2H), 2.20 (t, *J* = 6.94 Hz, 2H), 1.82 (pentet, *J* = 6.78 Hz, 2H), 1.25 (t, *J* = 7.25 Hz, 3H); ¹³C NMR (methanol-*d*₄) δ 177.3, 157.1, 60.8, 40.6, 35.7, 26.1, 14.7.

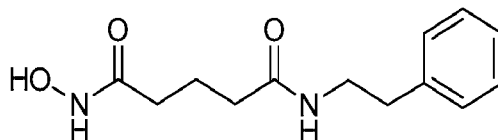


Ethyl 3-(phenethylcarbamoyl)propylcarbamate (santacruzamate A, 1): 4-

- ((ethoxycarbonyl)amino)butanoic acid (0.40 g, 2.28 mmol) was dissolved in CH₂Cl₂ (7 mL) and cooled to 0 °C. Phenethylamine (0.327 mL, 2.60 mmol) and triethylamine (0.64 mL, 4.56 mmol) were added to the solution followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.50 g, 2.60 mmol) in one portion. 4-Dimethylaminopyridine (catalytic (cat.)) was added and the solution was stirred at 0 °C for 60 min, then overnight at room temperature. The resulting solution was diluted with additional CH₂Cl₂ (20 mL) and washed sequentially with 10 mL of each of the following: 1.0 M HCl, sat. NaHCO₃, H₂O, brine. The organic layer was dried over Na₂SO₄ and concentrated to give a residue that was recrystallized by trituration with hexanes. Upon cooling to 0 °C the solution was filtered to yield **1**, which was obtained as a white solid (0.58 g, 92 % yield): mp 112-113 °C; IR ν_{max} (film) 3345, 3288, 1703, 1699, 1655, 1543, 1538, 1446, 1307, 1285, 1249, 1223, 1139, 1050, 1031; ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 2H), 7.20-7.28 (m, 3H), 5.96 (br s, 1H), 4.96 (br s, 1H), 4.12 (q, *J* = 6.94 Hz, 2H), 3.55 (q, *J* = 6.73 Hz, 2H), 3.20 (q, *J* = 5.88 Hz, 2H), 2.85 (t, *J* = 6.94 Hz, 2H), 2.20 (t, *J* = 6.94 Hz, 2H), 1.82 (pentet, *J* = 6.78 Hz, 2H), 1.25 (t, *J* = 7.25 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 157.1, 138.9, 128.8, 128.6, 126.5, 60.8, 40.6, 40.2, 35.7, 33.7, 26.1, 14.7; ESI-MS *m/z* (%) 301.1 (8, [M+Na]⁺), 280.2 (25) 279.3 (100, [M+H]⁺); HRESI-MS [M+H]⁺ *m/z* 279.1726 (calculated for C₁₅H₂₃N₂O₃, 279.1709).



Methyl 4-(phenethylcarbamoyl)butanoate: *mono*-Methyl glutarate (1.00 g, 6.84 mmol) was taken up in CH₂Cl₂ (12 mL) and cooled to 0 °C. Phenethylamine (0.95 mL, 7.53 mmol) and triethylamine (1.91 mL, 13.68 mmol) were added to the solution followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.44 g, 7.53 mmol) in one portion. 4-Dimethylaminopyridine (cat.) was added and the solution was stirred overnight at room temperature. The resulting mixture was diluted with additional CH₂Cl₂ (20 mL) and washed sequentially with 20 mL of each of the following: 1.0 M HCl, sat. NaHCO₃, H₂O, brine. The organic phase was then passed through Celite, dried over Na₂SO₄, and concentrated to yield 4-(phenethylcarbamoyl)butanoate (1.50 g, 6.02 mmol, 88 % yield) as a clear crystalline solid: mp 58.3-59.8 °C; ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 2H), 7.19-7.28 (m, 3H), 5.57 (br s, 1H), 3.68 (s, 3H), 3.52-3.57 (m, 2H), 2.84 (t, *J* = 6.94 Hz, 2H), 2.37 (t, *J* = 7.25 Hz, 2H), 2.18-2.23 (m, 2H), 1.95 (pentet, *J* = 7.25 Hz, 2H); ¹³C NMR (126 MHz, CDCl₃) δ 173.6, 172.0, 138.8, 128.6, 128.7, 126.5, 51.6, 40.5, 35.7, 35.5, 33.0, 20.8; HRESI-MS [*M*+H]⁺ *m/z* 250.1431 (calculated for C₁₄H₂₀NO₃, 250.1443).



N¹-hydroxy-N⁵-phenethylglutaramide (SCA-SAHA hybrid, 3): Hydroxylamine hydrochloride (5.55 g, 79.8 mmol) in methanol (150 mL) was mixed with KOH (4.48 g, 79.8 mmol) at 40 °C in methanol (22 mL), cooled to 0 °C, and filtered. The butyric acid methyl ester (1.08 g, 4.43 mmol) was then added to the filtrate followed by addition (over 30 min) of KOH (0.36 g, 6.49 mmol). The mixture was stirred at room temperature overnight. The resulting solution was concentrated and resuspended in 50 mL of ice cold H₂O and the pH was adjusted to 7.0 using acetic acid. The solution was cooled overnight at 0 °C to yield **3** (0.98 g, 4.13 mmol, 90%) as a white crystalline solid: mp 106-107 °C; IR *v*_{max} (film) 3302, 3177, 2920, 2360, 2340, 1623, 1617, 1565, 1458, 1419, 1195, 1033, 940; ¹H NMR (500 MHz, methanol-*d*₄) δ 7.30-7.37 (m, 2H), 5.52 (br s, 1H), 3.51-3.58 (m, 2H), 2.84 (t, *J* = 6.94 Hz, 2H), 2.37 (t, *J* = 7.25 Hz, 2H), 2.21 (t, *J* = 7.41 Hz, 2H), 1.96 (pentet, *J* = 7.25 Hz, 2H); ¹³C NMR (126 MHz, methanol-*d*₄) δ 173.8, 170.8, 139.1, 128.4, 128.1, 125.9, 40.6, 35.1, 34.8, 31.6, 21.7; ESI-MS *m/z* (%) 273.1 (8, [*M*+Na]⁺), 252.1 (15), 251.1 (100, [*M*+H]⁺), 218.2 (10); HRESI-MS [*M*+H]⁺ *m/z* 251.1370 (calculated for C₁₃H₁₉N₂O₃, 251.1396).

Biological Assays. All assays were run using established protocols or following kit directions. Brief experimental details are provided below.

Plasmodium falciparum (malaria) assay: *P. falciparum* malaria parasites are maintained and assayed in human erythrocytes, from chloroquine-resistant *P. falciparum* strain (Indochina W2). Cultures are maintained *in vitro* in type O+ human erythrocytes. The bioassay involves the use of synchronized ring form parasites that are incubated with extracts,
5 fractions, compounds, or controls (chloroquine is used as positive control) for 48 hours within a humidified, air-tight container, flushed with a specialized gas mixture (5% CO₂, 5% O₂, and 90% N₂). Parasite percent growth (%G) is measured using an aliquot of culture medium transferred to a new plate, permeabilized with Triton X, and treated with PicoGreen[®], a fluorescent nucleic acid stain for quantitating double-stranded DNA. The
10 bioassay measures parasite %G by determining the quantity of PicoGreen intercalated into intact parasitic DNA (erythrocytes are anucleate and so do not absorb PicoGreen).

HDAC Enzyme Assay: Three HDAC isozymes [HDAC2 (Class I), HDAC4 (Class Ia), and HDAC6 (Class IIb)] were utilized to determine percent inhibition and IC₅₀ values of SCA (1) and the SCA-SAHA hybrid (3), using commercially available human recombinant
15 enzyme (BPS Bioscience) and fluorogenic HDAC assay kits (HDAC2 kit from Active Motif; HDAC4 and HDAC6 kits from BPS Bioscience). SAHA (2, Vorinostat[®]; Sigma Aldrich, St. Louis, MO) served as a control for the enzyme inhibition assay. Assay data were subjected to non-linear regression analysis (GraphPad Software, Inc., CA). Enzyme inhibition assays were performed with varying concentrations of 1, 2, or 3. Briefly, components were added
20 sequentially to a black, flat bottom 96-well microtiter plate (Sigma-Aldrich) as described by the manufacturer's protocol and the reaction mixture was incubated for 30 minutes at 37 °C. The potent HDAC inhibitor, trichostatin A (included in the assay kit), was added to the bifunctional HDAC assay developer at a final reaction concentration of 1 µM to stop deacetylation and initiate the release of the fluorophore. The reaction mixture was further
25 incubated at room temperature for 15 minutes. Fluorescence was measured on a Spectra Max Gemini XPS (Molecular Devices, Sunnyvale CA) using an excitation wavelength of 360 nm and a detection wavelength of 460 nm. Inhibition assays were used to determine the half maximal inhibitory concentration, IC₅₀, in HDAC2.

Cell Cytotoxicity Assay: Human colon cancer cells (HCT-116) were obtained from
30 the American Type Culture Collection (ATCC, Manassas, VA) and cultivated according to supplier's instructions using McCoy's 5A media supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% non-essential amino acids. Upon subconfluent growth, cells were seeded in a 96-well plate at 5,000 cells per well. Before treatment, the

plates were incubated at 37 °C, 5% CO₂ for 24 hours. Treatment with test compounds was carried out in triplicate wells for 96 hours using SAHA (3) as a positive control.

Human cutaneous T lymphocyte (HuT-78) cells were obtained from ATCC and cultivated according to supplier's instructions using Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Cells were seeded at log growth phase at 50,000 cells per well. Plates were incubated at 37 °C, 5% CO₂ for 4 h, treated with compounds in triplicate, and incubated for 72 hours using SAHA (3) as a positive control.

Human dermal fibroblast cells (hDF) were obtained from ATCC and cultivated according to supplier's instructions using Dulbecco's Modified Eagle Medium (DMEM) F-12 media supplemented with 40% FBS and 1% penicillin/streptomycin. Cells were seeded at 5,000 cells per well and incubated at 37 °C, 5% CO₂ for 24 hours (adherent cells). Treatment with compounds was carried out in triplicate for 72 hours using SAHA (3) as a positive control.

IC₅₀ cell cytotoxicity values were determined by varying concentrations of all compounds and assay data was subjected to non-linear regression analysis (GraphPad Software, Inc., CA).

Results

A dark brown cyanobacterium, morphologically resembling the genus *Symploca* (strain PAC-19-FEB-10-1), was collected from a coral and rock reef near Santa Cruz Island during an expedition to the Coiba National Park on the Pacific coast of Panama. Microscopically, the specimen was composed of fine (9-10 µm wide) filaments with isodiametric cells covered with a barely visible sheath.

The SSU (16S) rRNA gene sequence was obtained from the strain PAC-19-FEB-10-1 (GenBank acc. nr. JX458089.1) and used to infer the evolution of this specimen in relation to other groups of cyanobacteria. This phylogenetic inference revealed that the closest related reference-strain was *Symploca atlantica* PCC 8002^R (GenBank acc. nr. AB039021). However, the uncorrected gene sequence divergence between this clade and the original type-strain was 4.5% over 1162 base pairs in the 16S rRNA gene. This high evolutionary divergence, in combination with distinct biogeographic and ecological divergence, suggested that strain PAC-19-FEB-10-1 should compose an independent group, distinct from the genus *Symploca*. Phylogenetically closely related to PAC-19-FEB-10-1 was the hoiamide C-producing strain PNG05-8 (GenBank acc. nr. HM072003) from Papua New Guinea (p-

distance = 0.1% gene sequence divergence). There is a second lineage of “tropical marine *Symploca*” (p-distance = 5.8% gene sequence divergence from the “tropical marine *Symploca*” clade containing PAC-19-FEB-10-1 and PNG05-8), which includes the dolastatin 10 producing strain VP642b (AY032933) and the symplostatin 1 and 2 producing strain
5 VP377 (AF306497). Thus, not only should “tropical marine *Symploca*” be separated from the current genus *Symploca*, but also, there are at least two different groups within the “tropical marine *Symploca*” which may be distinct from one another at the genus level.

A polar fraction from the initial normal phase flash chromatography of the crude extract, fraction H (eluted with 3:1 ethyl acetate:methanol), was tested at 10 µg/mL and
10 found to have potent activity against the malaria parasite (99.9% inhibition of parasite growth) and MCF-7 cancer cells (50% cell death indicated by negative growth, perhaps indicative of overt cytotoxicity), with no activity in leishmaniasis or Chagas’ disease assays (7.9% and 9.2% inhibition, respectively). Isolation efforts continued using reverse phase solid phase extraction (RP-SPE) and RP high performance liquid chromatography (HPLC),
15 and yielded a single molecule, santacruzamate A (SCA, **1**), with these pronounced biological properties.

By HRESIMS an $[M+H]^+$ peak consistent with a molecular formula of $C_{15}H_{22}N_2O_3$ was obtained, indicative of five degrees of unsaturation. 1H NMR analysis of the purified metabolite (**1**) revealed a relatively uncomplicated NMR spectrum with two amide protons
20 (δ_H 5.92 and 4.92), five phenyl protons (δ_H 7.22 – 7.30), six sets of methylene protons (δ_H 4.10, 3.53, 3.18, 2.83, 2.17, and 1.80), and one methyl group (δ_H 1.23). The dispersions in chemical shift and coupling patterns helped to determine the respective linkage of these protons in four distinct spin systems (**1a-1d**). The most deshielded methylene signal, a quartet at δ_H 4.10, was consistent with protons attached to a carbon bearing an oxygen atom
25 (by HSQC, δ_C 60.8), and proximal to a methyl group (for which the resonance was located by 1H - 1H COSY at δ_H 1.23), thus defining partial structure **1a**. Two of the methylene groups had 1H and ^{13}C NMR chemical shifts indicative of proximity to nitrogen atoms (δ_H 3.53 and 3.18), both of which resonated as quartets. Delineation of a second spin system (**1b**) began with the higher field of these quartets (δ_H 3.18; δ_C 40.2) which was coupled to both an amide
30 1H singlet at δ_H 5.92 and a methylene resonance at δ_H 1.80 (CH_2 -12, δ_C 26.1). The pentet splitting pattern of this latter signal suggested a third contiguous methylene group, and this was confirmed via COSY correlations between H_2 -12 and H_2 -11 (δ_H 2.17; δ_C 33.7) signals. The more downfield quartet for a methylene adjacent to nitrogen (CH_2 -8, δ_H 3.53 and δ_C

40.6) was proximate to another methylene (CH₂-7, δ_{H} 2.83, δ_{C} 35.7), thereby defining a –CH₂–CH₂– spin system (**1c**). The fourth spin system (**1d**) was represented by three overlapping ¹H multiplets in the aromatic region that integrated to five protons, as is typical of a monosubstituted phenyl group (Table 1). HMBC correlations from H-7 (δ_{H} 2.83) and H-8 (δ_{H} 3.53) to C-4 (δ_{C} 138.9) linked partial structures **1c** and **1d** to define a phenethylamine moiety. HMBC correlations from H-8, H-11 and H-12 to an amide carbonyl at δ_{C} 172.5 (C-10) established the connectivity of partial structures **1b** and **1c**, and further identified partial structure **1b** as a γ -aminobutyric acid residue. Finally, HMBC correlations from both H-13 and H-16 to an unusually shielded carbonyl at δ_{C} 157.1 (C-15) connected partial structures **1a** and **1b**. The distinctive chemical shift of this C-15 carbonyl, along with the remaining atoms required by the molecular formula, were consistent with a carbamate functionality spanning these two spin systems. The final, achiral molecular structure of santacruzamate A (**1**) was thus established.

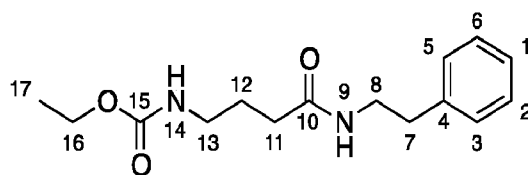
Table 1. NMR Data for Santacruzamate A (**1**) in CDCl₃.

position	$\delta_{\text{H}}^{\text{a}}$, mult. (<i>J</i> in Hz)	$\delta_{\text{C}}^{\text{b}}$, mult.	COSY	HMBC (H→C)
1	7.23, m	126.5, CH	2, 6	3, 5
2	7.30, m	128.8, CH	1, 3	4, 6
3	7.22, m	128.6, CH	2	1, 5, 7
4	-	138.9, qC	-	-
5	7.22, m	128.6, CH	6	1, 3, 7
6	7.30, m	128.8, CH	1, 5	2, 4
7	2.83, t (6.8)	35.7, CH ₂	8	3, 4, 5, 8
8	3.53, q (6.8)	40.6, CH ₂	7, 9	4, 7, 10
9	5.92, br s	-	8	-
10	-	172.5, qC	-	-
11	2.17, t (6.9)	33.7, CH ₂	12	10, 12, 13
12	1.80, pentet (6.8)	26.1, CH ₂	11, 13	10, 11, 13
13	3.18, q (5.9)	40.2, CH ₂	12, 14	11, 12, 15
14	4.92, br s	-	13	-
15	-	157.1, qC	-	-
16	4.10, q (6.9)	60.8, CH ₂	17	15, 17
17	1.23, t (7.3)	14.7, CH ₃	16	16

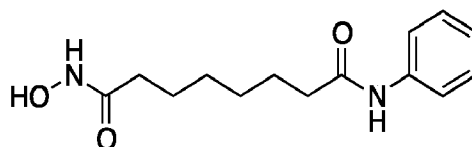
^a Measured at 400 MHz. ^b Measured at 100 MHz.

SCA (1) has several structural features in common with SAHA (2), a clinically approved histone deacetylase (HDAC) inhibitor used to treat refractory cutaneous T-cell lymphoma. The target of SAHA includes all isozyme sub-types of histone deacetylases (HDACs), the consequence of which is to upregulate the transcription of cell cycle regulators, nuclear transcription factors, and pro-apoptotic genes, thus bringing about an overall antineoplastic effect. Structural similarity to SCA can also be seen with two other marine natural products: psammaplin H (4), a potent HDAC inhibitor isolated from a marine sponge; and grenadamide (5) from a marine cyanobacterium with activity in a central nervous system (CNS) assay (see Scheme 12 for structures).

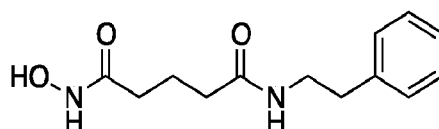
Scheme 12:



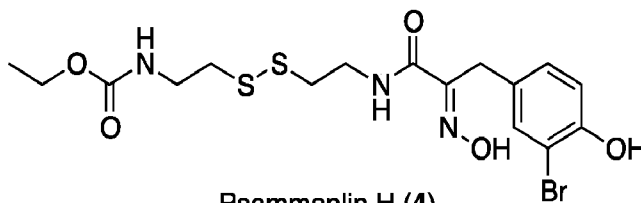
Santacruzamate A (SCA, 1)



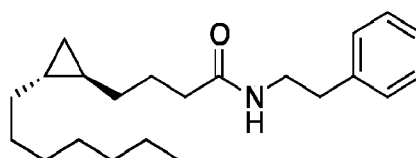
SAHA (2)



SCA-SAHA hybrid (3)



Psammaplin H (4)



Grenadamide (5)

SAHA binds to HDAC enzymes such that the phenyl cap sits above the enzyme pocket into which the aliphatic chain inserts, positioning to hydroxamic acid adjacent to the enzymatic zinc at the distal end of the pocket. The chemical syntheses of compound **1**, as well as a hybrid structure SCA and SAHA, were undertaken. In brief, GABA was converted to the carbamate intermediate, 4-((ethoxycarbonyl)amino)-butanoic acid via reaction with K_2CO_3 and ethyl chloroformate in water (76% yield). This intermediate was coupled to phenethylamine using *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC-HCl), triethylamine (TEA) and catalytic 4-(dimethylamino)pyridine (DMAP) to yield SCA (**1**, 92% yield, overall yield 70%). To create an SCA-SAHA hybrid structure (**3**, Scheme 13), phenethylamine was coupled to *mono*-methyl glutarate via the peptide coupling described above (88% yield), and then this intermediate was converted to the hydroxamic acid via standard literature procedures using hydroxylamine hydrochloride and KOH in methanol to afford compound **3** (90% yield, overall yield 79%).

The synthetic products **1** and **3**, as well as the original natural product SCA (**1**), were all evaluated for isozyme-selective inhibition of HDAC2, a Class I HDAC, and HDAC4, a Class IIa HDAC. Initial results revealed that compounds **1** and **3** at 1 μ M selectively inhibited HDAC2 with relatively little inhibition of HDAC4. At 1 μ M SAHA inhibits both Class I and II HDAC enzymes. Based on these promising results, IC_{50} values for the natural product SCA, synthetic SCA, the SCA-SAHA hybrid, and authentic SAHA were determined using HDAC2, HDAC4, and HDAC6, a Class IIb enzyme. To HDAC2, SAHA (**2**) showed an IC_{50} of 85.8 nM whereas SCA (**1**) and synthetic SCA (**1**) yielded values of 119 pM and 112 pM, respectively. Thus, SCA is over 700-fold more potent to HDAC2 than the clinically useful drug SAHA. The SCA-SAHA hybrid (**3**) was of diminished activity compared to SCA, with an IC_{50} of 3.5 nM against HDAC2. Both samples of SCA (**1**) and the SCA-SAHA (**3**) hybrid were found to have IC_{50} values over 1 μ M against HDAC4, indicating a strong selectivity for Class I HDAC inhibition. SCA (**1**) and SCA-SAHA (**3**) were further tested against HDAC6 and found to have IC_{50} values of 433.5 nM and 385.8 nM, respectively, while SAHA shows limited selectivity, with an IC_{50} of 38.9 nM (Table 2).

Table 2. Biological Activity of Santacruzamate (**1**) and Synthetic Compounds against Class I and Class II HDACs using HDAC2, HDAC4, and HDAC6 as well as in Cellular Cytotoxicity Testing using HCT-116 Colon Cancer, HuT-78 Cutaneous T-cell Lymphoma, and Human Dermal Fibroblast (hDF) Cells.

	HDAC2	HDAC4	HDAC6		HCT116	HuT-78	hDF
	IC ₅₀ (nM)	IC ₅₀ (nM)	IC ₅₀ (nM)	SI*	GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)
1 (natural product)	0.119	> 1000	433.5	3643	29.4	1.4	>100
1 (synthetic)	0.112	> 1000	433.4	3870	28.3	1.3	>100
3	3.5	> 1000	385.8	110	2.3	0.7	>100
2 (positive control)	85.8	n.d.	38.9	0.33	0.4	3.0	6.1

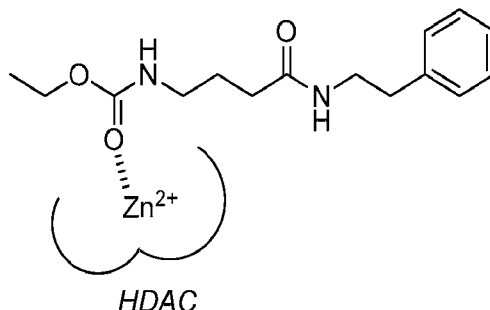
* SI = selectivity index calculated as HDAC6/HDAC2; n.d. = no data

Subsequently, the natural product SCA and synthetic compounds were tested for cytotoxicity to HCT-116 colon carcinoma cells, HuT-78 cutaneous T-cell lymphoma cells, and human dermal fibroblast (hDF) cells. SAHA was used as the positive control and showed a GI₅₀ value of 0.4 μM in HCT-116 cells, 3.0 μM in HuT-78 cells, and 6.1 μM in hDF cells (Table 2). SCA-SAHA was slightly less potent in HCT-116 cells (GI₅₀ of 2.3 μM) but of somewhat greater potency in HuT-78 cells (GI₅₀ 0.7 μM) with no activity against hDF cells (GI₅₀ >100 μM). The natural and synthetic SCA were found to be of only moderate potency in HCT-116 cells with GI₅₀ values of 29.4 and 28.3 μM, respectively. However, both potently inhibited the growth of HuT-78 cells with GI₅₀ values of 1.4 μM and 1.3 μM, respectively, and neither exhibited activity against hDF cells (GI₅₀ >100 μM).

Example 2: Compounds of Formula III - Zinc-Binding Group Analogs of Santacruzamate A

As described above in Example 1, a novel natural product, isolated from a dark brown cyanobacterium with a currently unconfirmed genus and species known as Santacruzamate A (SCA), has been discovered. This compound shows exceptional HDAC isoform selectivity (~3500:1 HDAC6/HDAC2) and picomolar activity in Class I: HDAC2 inhibition. Described herein are metal-binding domain (ethyl carbamate) analogs of SCA, leaving the three carbon aliphatic linker and phenethylamine cap group unaltered. Modifications consisted of substituting different carbamate moieties, conversion to N-amide derivatives, and insertion of known potent metal-binding ligands, with the purpose of exploring the electronic and spatial environment needed for the presumed monodendate coordination to Zn²⁺ (Scheme 13).

Scheme 13: Presumed Mono-dendate coordination of Santacruzamate A to Zn^{2+} in active site of HDAC2



Experimental:

5 *Chemistry. General.* All chemicals were used as received from Sigma-Aldrich or Acros without further purification. Hexanes, tetrahydrofuran (THF), diethyl ether (Et_2O), and dichloromethane (CH_2Cl_2) were used directly from a Baker cycle-tainer system. All glassware was flame-dried under vacuum, and all reactions were performed under an argon atmosphere, unless otherwise noted. Thin-layer chromatography was done on Fluka glass-backed TLC plates with fluorescent indicator and 0.2 mm silica gel layer thickness, and *p*-anisaldehyde was used as a developing agent. Column chromatography was done using 60 Å porosity, 32-63 μm silica gel. Melting points were collected on Mel-Temp digital melting point apparatus. NMR spectra were collected on a Brüker Avance 500 (500.13 MHz ^1H , 125.65 MHz ^{13}C) with chemical shifts given in ppm downfield from TMS. LCMS data were collected on a Agilent ESI single quadripole mass spectrometer coupled to an Agilent HPLC system with a G1311 quaternary pump, G1322 degasser, and a G1315 diode array detector using an Eclipse XDB-C₁₈ (4.6 x 150mm, 5 μm) RP-HPLC column. High Resolution Mass Spectra (HRMS) were collected on a Micromass VB-QTOF tandem mass spectrometer. Structural integrity and purity of the test compounds were determined by the composite of ^1H and ^{13}C NMR, melting point range, LCMS and HRMS and were found to have $\geq 95\%$ purity.

Experimental Data. General procedures for the synthesis of HDAC inhibitors, including intermediate and final compounds, and characterization data for the compounds are given below for the compounds of **Formula III**.

Synthesis of HDAC Inhibitors. General Procedure for the Preparation of
 25 **Intermediate Target Compounds.** γ -Aminobutyric acid (0.50 g, 4.85 mmol) was dissolved in H_2O (7 mL). Once dissolved K_2CO_3 (1.74 g, 12.6 mmol) was added and the resulting solution was cooled to 0°C . Respective chloroformate (6.31 mmol) was added dropwise and

the solution was stirred at 0 °C for 2 hours and then overnight at room temperature. The reaction mixture was then diluted with H₂O (20 mL) and extracted with EtOAc (3 x 10mL). The aqueous phase was acidified to pH = 2 with cold concentrated HCl and extracted with EtOAc (3 x 20 mL). The EtOAc extracts were dried over Na₂SO₄, concentrated to reveal a solid which was recrystallized from cold hexane to yield pure product.

4-(((Ethylthio)carbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; White solid (1.1 g, 90%); mp 84-85 °C. ¹H NMR (500 MHz, CDCl₃) δ 5.72 (br. s., 1H), 3.24-3.47 (m, 2H), 2.92 (q, J =7.57 Hz, 2H), 2.44 (t, J =7.09 Hz, 2H), 1.80-1.98 (m, 2H), 1.30 (t, J =7.41 Hz, 1H), 1.27 (s, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 178.6, 168.0, 40.5, 31.2, 24.7, 24.4, 15.7.

4-Methoxycarbonylamino butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; White solid (0.42 g, 81%); mp 38-40 °C ¹H NMR (500 MHz, CDCl₃) δ 11.01 (br. s., 1H), 6.26 (br. s., 1H), 3.57 (s, 3H), 3.08-3.19 (m, 2H), 2.32 (t, J =7.25 Hz, 2H), 1.75 (quin, J =7.09 Hz, 2H) ¹³C NMR (126 MHz, CDCl₃) δ 177.7, 157.5, 60.5, 52.1, 40.2, 31.1, 24.8, 20.9, 14.0.

4-((Propoxycarbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; White solid (0.67 g, 94%); mp 59-60 °C.

4-((Butoxycarbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; White solid (0.54 g, 89%); mp 48-49 °C.

4-(((Prop-2-yn-1-yloxy)carbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; mp = 48 °C.

4-(((2-Fluoroethoxy)carbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure; White solid (0.42 g, 89%); mp 62.1 °C.

4-(((Chloromethoxy)carbonyl)amino)butanoic acid. Converted to the carbamate derivative from γ -aminobutyric acid using the general procedure. The crude oil product was used immediately in the subsequent coupling without further purification.

4-tert-Butoxycarbonylamino butanoic acid. γ -Aminobutyric acid (1.00 g, 1.00 mmol) was dissolved in THF (15 mL) and 1M NaOH (10 mL) and cooled to 0°C. Boc₂O (2.66 g, 1.30 mmol) was added in one portion and the solution was stirred at 0°C for 2h and then overnight at room temperature. The solvent was evaporated and the residue was taken up

in H₂O (30 mL). The aqueous phase was washed EtOAc (3 x 10 mL) and then acidified with cold 1M HCl to pH 2. The solution was quickly extracted with EtOAc (3 x 30 mL) and the organic phase was dried over MgSO₄ and concentrated. The white residue was recrystallized from cold hexane to a white crystalline solid (1.98 g, 97 %): mp 56.0 - 57.5 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 1.44 (s, 9H), 1.82 (quint, *J* = 6.96 Hz, 2H), 2.40 (t, *J* = 6.33 Hz, 2H), 3.18 (q, *J* = 6.33 Hz, 2H), 4.67 (br s, 1 H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 172.5, 138.9, 128.8, 128.6, 126.5, 79.3, 40.6, 39.7, 35.6, 33.7, 28.4.

4-(Phenylmethoxy)carbonylamino butanoic acid. γ-Aminobutyric acid (0.10 g, 0.97 mmol) was dissolved in H₂O (4.8mL) and cooled to 0 °C. NaHCO₃ (0.25 g, 2.45 mmol) was added followed by dropwise addition of benzyl chloroformate (0.28 g, 1.06 mmol) dissolved in dioxane (2.9 mL). The solution was stirred for three hours at 0 °C then overnight at room temperature. The resulting solution was diluted with H₂O (10mL) adjusted and extracted with ethyl acetate (1 x 10 mL). The aqueous layer was adjusted to pH 2 with conc. HCl and extracted with ethyl acetate (3 x 30mL). The organic phase was washed with H₂O (2 x 10 mL) and brine (1 x 10mL) and dried over Na₂SO₄ and evaporated to dryness. The resulting residue was recrystallized from ethyl acetate/hexane to yield a white solid (0.25 g, 85%). Mp 60-62 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 7.23-7.44 (m, 5H), 5.08 (s, 2H), 3.17 (t, *J* = 6.94 Hz, 2H), 2.34 (t, *J* = 7.41 Hz, 2H), 1.80 (quin, *J* = 7.17 Hz, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 175.6, 157.5, 137.0, 128.1, 127.6, 127.4, 66.0, 39.8, 30.7, 24.9.

4-Fmoc-amino butanoic acid. Converted to the carbamate derivative from γ-aminobutyric acid using the general procedure; White solid (0.51 g, 71%); mp 166-167 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 7.80 (d, *J* = 7.57 Hz, 2H), 7.65 (d, *J* = 7.25 Hz, 2H), 7.36-7.44 (m, 2H), 7.28-7.36 (m, 2H), 4.90 (br. s., 2H), 4.36 (d, *J* = 6.94 Hz, 2H), 4.15-4.28 (m, 1H), 3.16 (t, *J* = 6.62 Hz, 2H), 2.27-2.38 (m, 2H), 1.70-1.84 (m, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 175.6, 157.5, 143.9, 141.2, 127.3, 126.7, 124.7, 119.5, 66.2, 39.7, 30.7, 24.9.

4-(p-Nitrobenzyloxycarbonyl)amino butanoic acid. Converted to the carbamate derivative from γ-aminobutyric acid using the general procedure; White solid (0.321 g, 73%); mp 143-145 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 8.29 (d, *J* = 9.14 Hz, 2H), 7.22-7.55 (m, 2H), 3.22-3.32 (m, 2H), 2.38-2.49 (m, 2H), 1.84-1.97 (m, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 175.5, 156.3, 154.3, 144.7, 124.6, 122.0, 40.1, 30.7, 24.6.

4-(Allyloxycarbonyl)amino butanoic acid. Converted to the carbamate derivative from γ-aminobutyric acid using the general procedure; White solid (0.74 g, 83%); mp 43-44 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 5.90-6.01 (m, 1H), 5.31 (dd, *J* = 1.42, 17.18 Hz, 1H),

5.19 (d, $J=10.40$ Hz, 1H), 4.90 (br. s., 1H), 4.54 (d, $J=5.36$ Hz, 2H), 3.17 (t, $J=6.78$ Hz, 2H), 2.35 (t, $J=7.41$ Hz, 2H), 1.80 (quin, $J=7.17$ Hz, 2H). ^{13}C NMR (126 MHz, methanol- d_4) δ 175.6, 157.4, 133.1, 116.0, 64.9, 39.7, 30.7, 24.9.

4-*p*-Tolylsulphonylethoxyamino butanoic acid. 2-*p*-Tolylsulphonylethyl

5 chloroformate (0.56 g, 2.14 mmol) was dissolved in THF (4 mL) and added dropwise to a 0°C suspension of γ -aminobutyric acid (0.22 g, 2.14 mmol) and magnesium oxide (0.13 g, 3.21 mmol) in H_2O (9.4 mL). The mixture was stirred for 1 hour at 0 °C and then at room temperature for an additional 1 hour. The mixture was acidified using conc. HCl to pH 2 and extract with CH_2Cl_2 (3 x 20 mL), dried over Na_2SO_4 and concentrated in vacuo. The resulting
10 residue was triturated with hexane, cooled and filtered to give a white solid (0.69 g, 81%); mp 109-110 °C. ^1H NMR (500 MHz, methanol- d_4) δ 7.82 (dd, $J=4.89$, 8.04 Hz, 3H), 7.45 (d, $J=7.57$ Hz, 3H), 4.33 (t, $J=5.99$ Hz, 2H), 3.87 (t, $J=6.31$ Hz, 1H), 3.57 (t, $J=5.83$ Hz, 2H), 3.41 (t, $J=6.31$ Hz, 1H), 3.06 (t, $J=6.94$ Hz, 2H), 2.47 (s, 3H), 2.44-2.44 (m, 1H), 2.29 (t, $J=7.41$ Hz, 2H), 1.67-1.75 (m, 2H). ^{13}C NMR (126 MHz, methanol- d_4) δ 175.5, 156.4, 145.1,
15 136.6, 129.7, 129.6, 127.8, 127.8, 57.9, 55.4, 55.0, 39.7, 30.6, 24.8, 20.3.

4-Acetamido butanoic acid. γ -Aminobutyric acid (0.50 g, 4.85 mmol) was dissolved in acetic acid (12 mL) and then acetic anhydride (1.47 mL, 15.7 mmol) and diethyl ether (7.3 mL) were added dropwise. The mixture was stirred for 1 hour then evaporated to dryness and recrystallized from cold aqueous solution. The crystalline solid was suspended in toluene and
20 concentrated in vacuo three times. The product obtained was washed with hexane to yield a white crystalline solid (1.41 g, 95%); mp 130-131 °C. ^1H NMR (500 MHz, methanol- d_4) δ 3.22 (t, $J=7.09$ Hz, 2H), 2.35 (t, $J=7.41$ Hz, 2H), 1.95 (s, 3H), 1.80 (quin, $J=7.17$ Hz, 2H). ^{13}C NMR (126 MHz, methanol- d_4) δ 175.5, 172.0, 38.4, 30.8, 24.4, 21.1.

4-Propionamidobutanoic acid. γ -Aminobutyric acid (0.50 g, 4.85 mmol) was
25 dissolved in propionic anhydride (1 mL) and then cat. sulfuric acid (0.1 mL) was added and the mixture was heated at 100 °C for 2.5 hrs. The solution was cooled to room temperature and the resulting crystalline solid was collected via filtration. The solid was washed with ether (5 x 20 mL) to yield a shimmering white solid (0.981 g, 74%) 87-89 °C. ^1H NMR (500 MHz, methanol- d_4) δ 3.23 (t, $J=6.94$ Hz, 2H), 2.34 (t, $J=7.41$ Hz, 2H), 2.21 (q, $J=7.57$ Hz, 2H), 1.80 (quin, $J=7.17$ Hz, 2H), 1.14 (t, $J=7.72$ Hz, 3H). ^{13}C NMR (126 MHz, methanol- d_4)
30 δ 175.7, 38.4, 31.0, 28.8, 24.5, 9.1.

4-Butyramidobutanoic acid. γ -Aminobutyric acid (0.50 g, 4.85 mmol) was dissolved in butyric anhydride (1 mL) and then cat. sulfuric acid (0.1 mL) was added and the

mixture was refluxed 1 hr and then at 100 °C for an additional 2 hrs. The solution was cooled to room temperature and extracted with ether (3 x 20 mL) and concentrated. The resulting oil was purified with flash column chromatography using 100% EtOAc to yield a white solid (0.79 g, 88%) 69.0–70.0 °C. ¹H NMR (500 MHz, CDCl₃) δ 10.90 (br. s., 1H), 3.30 (q, *J*=6.62 Hz, 2H), 2.38 (t, *J*=7.09 Hz, 2H), 2.17 (t, *J*=7.57 Hz, 2H), 1.83 (quin, *J*=6.94 Hz, 2H), 1.63 (sxt, *J*=7.44 Hz, 2H), 0.92 (t, *J*=7.41 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 177.1, 174.5, 38.9, 38.4, 31.6, 24.5, 19.2, 13.7.

4-(3,3-Dimethylbutanoylamino) butanoic acid. γ-Aminobutyric acid (0.50 g, 4.85 mmol) was dissolved in H₂O (2 mL) of a 4N NaOH solution. 1,4-dioxane (2 mL) was added and the solution was cooled to 0 °C. Dropwise 3,3-dimethylbutyryl chloride was added with the concurrent addition of 4N NaOH to maintain the initial pH of 9-11. The solution was stirred for 2 hours at 0 °C and then overnight at room temperature. The resulting solution was diluted with H₂O (10mL) and acidified with conc. HCl to pH 2 and extracted with ethyl acetate (3 x 30 mL). The organic phase was washed with H₂O (1 x 10 mL) and brine (1 x 10 mL), dried over Na₂SO₄, and concentrated in vacuo to yield a crude oil which was used in the next reaction without further purification.

4-Benzamido butanoic acid. γ-Aminobutyric acid (0.30 g, 2.91 mmol) and NaOH (0.21 g, 5.24 mmol) were dissolved in H₂O (6 mL). The resulting solution was cooled to 0 °C and to this was added dropwise benzoyl chloride (0.31 mL, 2.63 mL). The mixture was stirred for two hours at 0 °C and then at room temperature overnight. The resulting solution was acidified with conc. HCl to pH 2, extracted with ethyl acetate (3 x 30mL), dried over Na₂SO₄, and concentrated in vacuo. The residue was taken up in minimal ethanol followed by the addition of water until a cloudy solution was formed. The solution was concentrated to yield a white solid which was triturated with hexane and filtered to yield a crystalline solid (1.41 g, 94%); mp 88-89 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 7.79-7.87 (m, *J*=0.95, 8.51 Hz, 1H), 7.50-7.56 (m, 2H), 7.43-7.50 (m, 3H), 3.45 (t, *J*=6.94 Hz, 2H), 2.41 (t, *J*=7.25 Hz, 2H), 1.94 (quin, *J*=7.17 Hz, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 182.1, 137.5, 136.9, 129.4, 128.3, 42.5, 36.1, 24.8, 21.3.

4-(3-(Acetylthio)propanamido)butanoic acid. γ-Aminobutyric acid (1.0 g, 9.69 mmol) and Na₂CO₃ (0.75 g, 7.07 mmol) were dissolved in H₂O (20 mL). Ethyl acetate (5 mL) was added and the solution was cooled to 0 °C. *S*-(3-chloro-3-oxopropyl) ethanethioate (made via standard literature procedure from thioacetic acid and acrylic acid) (1.61 g, 9.69 mmol) was added dropwise at 0 °C. During this time additional Na₂CO₃ was added in small

portions to maintain the pH > 9. The resulting mixture upon complete addition of *S*-(3-chloro-3-oxopropyl) ethanethioate, was stirred for 2 hrs at 0 °C and then at room temperature overnight. The reaction mixture was extracted with ethyl acetate (3 x 10 mL) and the aqueous layer acidified to pH = 2 with 6N HCl and was reextracted with EtOAc (3 x 20 mL).

5 The organic layer was washed with brine (1 x 20 mL), dried over Na₂SO₄ and concentrated. The resulting tan oil was purified via flash column chromatography in 100% EtOAc yielding 4-(3-(acetylthio)propanamido)butanoic acid as an off-white solid (0.60 g, 27.0 %) Mp = 70.0 – 71.0 °C. ¹H NMR (500 MHz, CDCl₃) δ 10.55 (br. s., 1H), 6.34 (br. s., 1H), 3.33 (q, *J*=6.62 Hz, 2H), 3.14 (t, *J*=7.09 Hz, 2H), 2.51 (t, *J*=6.94 Hz, 2H), 2.42 (t, *J*=7.09 Hz, 2H), 2.34 (s, 3H), 1.86 (quin, *J*=6.94 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 196.6, 177.6, 171.5, 39.0, 36.2, 31.4, 30.6, 25.0, 24.5.

4-(Tosylamino) butanoic acid. γ-Aminobutyric acid (0.50 g, 4.85 mmol) and NaOH (0.78 g, 19.4 mmol) were dissolved in H₂O (12 mL). The solution was cooled to 0 °C and diethyl ether (1.0 mL) was added followed by TsCl (1.38 g, 7.27 mmol) portionwise. The solution was stirred vigorously for 2 hours at 0 °C and then stirred for one additional hour at room temperature. The reaction mixture was extracted with ether (1 x 20 mL) and acidified with conc. HCl to pH 2. The precipitate was collected by filtration and recrystallized from aqueous ethanol to yield a crystalline solid (0.51 g, 94%); mp 134-135 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 7.74 (d, *J*=7.88 Hz, 2H), 7.39 (d, *J*=7.88 Hz, 2H), 3.33 (td, *J*=1.69, 3.23 Hz, 1H), 2.89 (t, *J*=6.78 Hz, 2H), 2.44 (s, 3H), 2.33 (t, *J*=7.25 Hz, 2H), 1.74 (quin, *J*=7.17 Hz, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 175.7, 169.0, 134.3, 131.2, 128.2, 126.9, 39.0, 31.0, 24.5.

4-(2-Nitro-benzenesulfonyl)amino butanoic acid. Converted to the 2-nitro-benzenesulfonyl amide derivative using the procedure described for 4-(tosylamino) butanoic acid and was obtained as an off-white solid (0.28 g, 45 %); mp 143-145 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 8.06-8.14 (m, 1H), 7.79-7.91 (m, 3H), 3.09-3.16 (m, 2H), 2.35 (t, *J*=7.41 Hz, 2H), 1.76-1.85 (m, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 175.3, 148.2, 133.6, 133.4, 132.2, 130.2, 124.5, 42.2, 30.2, 24.7.

4-(2-Oxo-pyrrolidine-1-thiocarbonylamino) butanoic acid. 1,3-Di-(γ-carboxypropyl)-thiourea (0.80 g, 3.22 mmol) and *p*-toluenesulfonic acid monohydrate (0.08 g, 0.32 mmol) were combined and heated to 185 °C in vacuo (30 torr) for 15 minutes. After the melt had cooled it was crystallized from H₂O to yield a white solid (0.29 g, 38%); mp 127-128 °C. ¹H NMR (500 MHz, methanol-*d*₄) δ 4.15-4.19 (m, 2H), 3.67-3.72 (m, 2H), 3.33

(td, $J=1.69, 3.23$ Hz, 2H), 2.72 (t, $J=8.04$ Hz, 2H), 2.38 (t, $J=7.41$ Hz, 2H), 2.00-2.07 (m, 2H), 1.96 (quin, $J=7.25$ Hz, 2H). ^{13}C NMR (126 MHz, methanol- d_4) δ 180.8, 177.1, 175.3, 51.0, 44.0, 33.7, 30.8, 23.3, 16.4.

4-(2-Oxo-pyrrolidine-1-carbonylamino) butanoic acid. 1,3-Di-(γ -carboxypropyl)-urea (0.25 g, 1.08 mmol) and *p*-toluenesulfonic acid monohydrate (0.03 g, 0.13 mmol) were combined and heated to 185 °C in vacuo (10 torr) until bubbling ceased (approx. 15 minutes). After the melt had cooled it was crystallized from H₂O to yield a white solid (0.09 g, 48%); mp 116-117 °C. ^1H NMR (500 MHz, methanol- d_4) δ 3.79-3.84 (m, 2H), 3.31-3.35 (m, 3H), 2.62 (t, $J=8.20$ Hz, 2H), 2.36 (t, $J=7.41$ Hz, 2H), 2.01-2.09 (m, 2H), 1.85 (quin, $J=7.17$ Hz, 2H). ^{13}C NMR (126 MHz, methanol- d_4) δ 177.8, 175.4, 153.7, 45.4, 38.6, 32.6, 30.7, 24.7, 16.5.

4-(Dimethylamino)butanoic acid-HCl. γ -Aminobutyric acid (1.00 g, 9.69 mmol) was dissolved in 90% formic acid (2.52 mL) and 37% formaldehyde (1.94 mL) and refluxed for 16 hrs. The resulting solution was cooled, acidified with conc. HCl, and evaporated to dryness. The resulting solid was azeotroped with toluene several times to remove all traces of formic acid. The solid was then recrystallized from acetonitrile to yield 4-(dimethylamino)butanoic acid as the HCl salt. mp 148-149 °C.

Synthesis of HDAC Inhibitors. General Procedure for the Preparation of Final Target Compounds. The intermediate free acid (2.28 mmol) was dissolved in CH₂Cl₂ (7 mL) and cooled to 0 °C. Phenethylamine (2.60 mmol) and triethylamine (4.56 mmol) were added to the solution followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.50 g, 2.60 mmol) in one portion. 4-dimethylaminopyridine (cat.) was added and the solution was stirred at 0 °C for 60 minutes then overnight at room temperature. The resulting solution was diluted with additional CH₂Cl₂ (20 mL) and sequentially washed with 10 mL of each of the following: 1.0 M HCl, H₂O, sat. NaHCO₃, H₂O, brine. The organic layer was dried over Na₂SO₄ and concentrated to give a residue which was pushed through a plug of silica (100% ethyl acetate), titrated with hexane then recrystallized from ethyl acetate/hexane. Upon cooling to 0 °C, the solution was filtered to yield pure product.

Ethyl 3-(phenethylcarbamoyl)propylcarbamate (III-1). Was obtained as a white solid (0.58 g, 92%); mp 112-113 °C. ^1H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 2H), 7.20-7.28 (m, 3H), 5.96 (br. s., 1H), 4.96 (br. s., 1H), 4.12 (q, $J=6.94$ Hz, 2H), 3.55 (q, $J=6.73$ Hz, 2H), 3.20 (q, $J=5.88$ Hz, 2H), 2.85 (t, $J=6.94$ Hz, 2H), 2.20 (t, $J=6.94$ Hz, 2H), 1.82 (quin, $J=6.78$ Hz, 2H), 1.25 (t, $J=7.25$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl₃) 172.5, 157.1,

138.9, 128.8, 128.6, 126.5, 60.8, 40.6, 40.2, 35.7, 33.7, 26.1, 14.7 ESI-MS m/z (%) 301.1 (8, [M+Na]⁺), 280.2 (25) 279.3 (100, [M+H]⁺); HRESI-MS [M+H]⁺ (M + H)⁺ calculated for C₁₅H₂₃N₂O₃, 279.1709, obs. 279.1726.

Methyl 3-(phenethylcarbamoyl)propylcarbamate (III-2). Was obtained as a white solid (0.35 g, 94%); mp 109-110 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 1H), 7.20-7.28 (m, 1H), 5.96 (br. s., 1H), 4.96 (br. s., 1H), 4.12 (q, J =6.94 Hz, 1H), 3.67 (s, 3H), 3.20 (q, J =5.88 Hz, 1H), 2.85 (t, J =6.94 Hz, 1H), 2.20 (t, J =6.94 Hz, 1H), 1.82 (quin, J =6.78 Hz, 1H), 1.25 (t, J =7.25 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.7, 157.5, 139.3, 128.5, 127.7, 125.8, 51.9, 40.8, 35.9, 35.2, 32.7. HRMS-ESI (m/z): (M + H)⁺ calculated for C₁₄H₂₁N₂O₃, 265.1552, obs. 265.1543.

tert-Butyl (4-oxo-4-(phenethylamino)butyl)carbamate (III-3) (0.31 g, 75%); mp 94-95 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.37 (m, 2H), 7.19-7.28 (m, 3H), 6.06 (br. s., 1H), 4.76 (br. s., 1H), 3.55 (q, J =6.94 Hz, 2H), 3.15 (q, J =6.31 Hz, 2H), 2.85 (t, J =6.94 Hz, 2H), 2.18 (t, J =7.25 Hz, 2H), 1.80 (quin, J =6.78 Hz, 2H), 1.46 (s, 9H). ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 138.9, 128.8, 128.6, 126.5, 79.3, 40.6, 39.7, 35.6, 33.7, 28.4, 26.4. HRMS-ESI (m/z): (M + H)⁺ calculated for C₁₇H₂₇N₂O₃, 307.2022, obs. 307.2012.

N-(3-(Phenethylcarbamoyl)propyl)but-3-enamide (III-6). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from diethyl ether / hexane and was obtained as a white solid (0.47 g, 81%); mp 90.0-91.0 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.36 (m, 2H), 7.18-7.27 (m, 3H), 6.04 (br. s., 1H), 5.86-5.98 (m, 1H), 5.31 (qd, J =1.48, 17.30 Hz, 1H), 5.22 (dd, J =1.42, 10.56 Hz, 1H), 5.17 (br. s., 1H), 4.56 (d, J =5.36 Hz, 2H), 3.49-3.58 (m, 2H), 3.20 (q, J =6.31 Hz, 2H), 2.84 (t, J =7.09 Hz, 2H), 2.19 (t, J =6.94 Hz, 2H), 1.82 (quin, J =6.78 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 138.8, 128.8, 128.6, 126.5, 40.7, 35.6, 33.8, 25.5, 24.3, 15.7. HRMS-ESI (m/z): (M + H)⁺ calculated for C₁₆H₂₃N₂O₃, 291.1709, obs. 291.1717.

S-Ethyl (4-oxo-4-(phenethylamino)butyl)carbamothioate (III-10). Was obtained as a white solid (0.51 g, 84%); mp 118-119 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.35 (m, 2H), 7.19-7.27 (m, 3H), 5.84-6.09 (m, 1H), 3.52-3.58 (m, 2H), 2.91 (q, J =7.25 Hz, 2H), 2.85 (t, J =7.09 Hz, 2H), 2.20 (t, J =6.78 Hz, 2H), 1.84 (quin, J =6.62 Hz, 2H), 1.30 (t, J =7.41 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 138.8, 128.8, 128.6, 126.5, 40.7, 35.6, 33.8, 25.5, 24.3, 15.7. HRMS-ESI (m/z): (M + H)⁺ calculated for C₁₅H₂₃N₂O₂S, 295.1480, obs. 295.1464.

Benzyl 3-(phenethylcarbamoyl)propylcarbamate (III-13). Was obtained as a white solid (0.14 g, 95%); mp 115-116 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.39 (m, 6H), 7.17-7.28 (m, 4H), 5.91 (br. s., 1H), 5.10 (s, 2H), 3.53 (q, *J*=6.73 Hz, 2H), 3.22 (q, *J*=6.31 Hz, 2H), 2.83 (t, *J*=7.09 Hz, 2H), 2.18 (t, *J*=6.94 Hz, 2H), 1.77-1.86 (m, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 155.9, 139.1, 136.1, 128.9, 128.1, 127.7, 127.1, 126.5, 67.1, 40.9, 40.6, 35.3, 33.6, 26.2. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₂₀H₂₅N₂O₃, 341.1865, obs. 341.1832.

4-Nitrophenyl (4-oxo-4-(phenethylamino)butyl)carbamate, (III-16). Was obtained as a white solid (0.31 g, 76%); mp 151-152 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.29 (d, *J*=9.14 Hz, 2H), 7.51-7.62 (m, 2H), 7.22-7.55 (m, 5H), 6.11 (br. s., 1H), 4.57 (br. s., 1H), 3.15 (q, *J*=6.31 Hz, 2H), 2.85 (t, *J*=6.94 Hz, 2H), 2.18 (t, *J*=7.25 Hz, 2H), 1.86 (quin, *J*=6.78 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 157.6, 153.4, 144.5, 139.4, 128.6, 127.5, 125.5, 122.3, 40.6, 40.1, 35.1, 33.3. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₉H₂₂N₃O₅, 372.1559, obs. 372.1547.

(9H-Fluoren-9-yl)methyl 3-(phenethylcarbamoyl)propylcarbamate (III-18). Was obtained as a white solid (0.28 g, 65%); mp 102-103 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.41 (m, 5H), 7.18-7.28 (m, 3H), 5.86 (br. s., 1H), 5.11 (s, 2H), 3.50-3.58 (m, 2H), 3.23 (q, *J*=6.31 Hz, 2H), 2.84 (t, *J*=6.94 Hz, 2H), 2.19 (t, *J*=6.94 Hz, 2H), 1.83 (quin, *J*=6.78 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.5, 157.8, 143.6, 142.7, 139.1, 128.8, 127.7, 126.7, 125.1, 120.5, 67.6, 47.1, 40.8, 39.7, 35.6, 33.8, 26.1. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₂₇H₂₉N₂O₃, 429.2178, obs. 429.2170.

2-(Phenylsulfonyl)ethyl 3-(phenethylcarbamoyl)propylcarbamate (III-17). Was obtained as a white solid (0.22 g, 88%); Mp = 103-104 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.82 (dd, *J*=4.89, 8.04 Hz, 3H), 7.45 (d, *J*=7.57 Hz, 3H), 7.33-7.35 (m, 2H), 7.19-7.28 (m, 3H), 4.35 (t, *J*=5.99 Hz, 2H), 3.86 (t, *J*=6.31 Hz, 1H), 3.57 (t, *J*=5.83 Hz, 2H), 3.41 (t, *J*=6.31 Hz, 2H), 3.27 (q, *J*=6.31 Hz, 2H), 3.06 (t, *J*=6.94 Hz, 2H), 2.86 (t, *J*=7.25 Hz, 2H), 2.47 (s, 3H), 2.29 (t, *J*=7.41 Hz, 2H), 1.67-1.75 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 157.1, 145.1, 139.4, 136.6, 129.7, 129.6, 128.6, 127.8, 127.1, 57.9, 55.4, 55.0, 40.6, 39.7, 35.1, 30.6, 24.8, 20.3. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₂₂H₂₉N₂O₅S, 433.1797, obs. 433.1786.

4-Amino-N-phenethylbutanamide (III-11). Benzyl 3-(phenethylcarbamoyl)propylcarbamate (48.5 mg, 0.14 mmol) was dissolved in degassed methanol and 10% Pd/C (20 mg) was added in one portion under 1 atm of H₂. The reaction

was stirred for 8 hrs at room temperature and then filtered through Celite. The filtrate was concentrated and the resulting clear oil washed with hexane (3 x 5 mL) which yielded a clear amorphous semi-solid (32.1 mg, 85%). ¹H NMR (500 MHz, methanol-*d*₄) δ 7.26-7.33 (m, 2H), 7.16-7.26 (m, 3H), 4.92 (s, 1H), 4.84 (s, 1H), 3.43 (t, *J*=7.25 Hz, 2H), 2.81 (t, *J*=7.25 Hz, 2H), 2.67 (t, *J*=7.25 Hz, 2H), 2.23 (t, *J*=7.41 Hz, 2H), 1.76 (quin, *J*=7.33 Hz, 2H). ¹³C NMR (126 MHz, methanol-*d*₄) δ 174.0, 139.1, 128.4, 128.1, 126.0, 40.5, 40.2, 35.1, 32.9, 27.5. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₂H₁₉N₂O, 207.1497, obs. 207.1502.

4-(Dimethylamino)-N-phenethylbutanamide (III-13). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: 3.5 eq of triethylamine and was obtained as an oil (0.05 g, 43%). ¹H NMR (500 MHz, CDCl₃) δ 7.42-7.40 (m, 2H), 7.29-7.26 (m, 3H), 4.84 (s, 1H), 3.43 (t, *J*=7.25 Hz, 1H), 2.82 (t, *J*=7.24 Hz, 1H), 2.66 (t, *J*=7.25 Hz, 1H), 2.26 (s, 6H), 2.22 (t, *J*=7.40 Hz, 1H), 1.76 (quin, *J*=7.33 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 141.11, 128.6, 127.5, 125.6, 60.9, 47.3, 40.8, 35.2, 24.0. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₄H₂₂N₂O, 234.1732, obs. 234.1743.

4-Acetamido-N-phenethylbutanamide (III-14). Was obtained as a white solid (0.11 g, 75%); mp 112-113 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.33-7.35 (m, 2H), 7.19-7.28 (m, 3H), 3.53-3.59 (m, 2H), 3.53-3.59 (m, 2H), 3.27 (q, *J*=6.31 Hz, 2H), 2.86 (t, *J*=7.25 Hz, 2H), 2.21 (d, *J*=6.31 Hz, 2H), 2.00 (s, 3H), 1.77-1.88 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 174.4, 172.8, 138.9, 128.7, 128.6, 126.5, 40.7, 38.9, 35.7, 34.9, 29.8, 23.5. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₄H₂₁N₂O₂, 249.1603, obs. 249.1581.

N-Phenethyl-4-propionamidobutanamide (III-15). Was obtained as a white solid (0.52 g, 72%); mp 117-118 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.35 (m, 2H), 7.19-7.26 (m, 3H), 6.32 (br. s., 1H), 6.23 (br. s., 1H), 3.50-3.57 (m, 2H), 3.26 (q, *J*=5.99 Hz, 2H), 2.85 (t, *J*=7.09 Hz, 2H), 2.17-2.25 (m, 5H), 1.81 (quin, *J*=6.62 Hz, 2H), 1.16 (t, *J*=7.57 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 174.4, 172.8, 138.9, 128.7, 128.6, 126.5, 40.7, 38.9, 35.7, 33.9, 29.8, 25.5, 9.9. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₅H₂₃N₂O₂, 263.1760, obs. 263.1784.

4-Butyramido-N-phenethylbutanamide (III-16). Was obtained as a white solid (0.66 g, 84%); mp 119-120 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.35 (m, 1H), 7.19-7.26 (m, 1H), 6.35 (br. s., 1H), 6.22 (br. s., 1H), 3.50-3.56 (m, 1H), 3.26 (q, *J*=5.99 Hz, 1H), 2.85 (t, *J*=7.25 Hz, 1H), 2.12-2.24 (m, 2H), 1.81 (quin, *J*=6.62 Hz, 1H), 1.67 (sxt, *J*=7.44 Hz, 1H),

0.96 (t, $J=7.41$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.7, 172.8, 138.9, 128.7, 128.6, 126.5, 40.7, 38.9, 38.7, 35.7, 33.9, 25.6, 19.2, 13.8. HRMS-ESI (m/z): ($\text{M} + \text{H}$) $^+$ calculated for $\text{C}_{16}\text{H}_{25}\text{N}_2\text{O}_2$, 277.1916, obs. 277.1948.

4-(3,3-Dimethylbutanoylamino)-N-phenethylacetamide (III-17). Was obtained as
5 a white solid (1.32 g, 89%): Mp = 87-88 °C. ^1H NMR (500 MHz, CDCl_3) δ 7.27-7.35 (m, 2H), 7.18-7.26 (m, 3H), 6.44 (br. s., 1H), 6.17 (br. s., 1H), 3.48-3.57 (m, 2H), 3.24 (q, $J=6.20$ Hz, 2H), 2.85 (t, $J=7.09$ Hz, 2H), 2.20 (t, $J=6.78$ Hz, 2H), 2.05 (s, 2H), 1.80 (quin, $J=6.62$ Hz, 2H), 1.04 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ 172.8, 172.4, 138.9, 128.7, 128.6, 126.5, 50.6, 40.7, 38.8, 35.6, 33.9, 30.8, 29.9, 25.7. HRMS-ESI (m/z): ($\text{M} + \text{H}$) $^+$ calculated for
10 $\text{C}_{18}\text{H}_{29}\text{N}_2\text{O}_2$, 305.2229, obs. 305.2214.

4-(Acrylamide)-N-phenethylbutamide (III-18). Was obtained as a white solid (0.53 g, 41%): Mp = 90-91 °C. ^1H NMR (500 MHz, CDCl_3) δ 7.31-7.38 (m, 2H), 7.19-7.28 (m, 3H), 6.23-6.34 (m, 1H), 6.07-6.16 (m, 1H), 6.00 (br. s., 1H), 5.67 (dd, $J=1.42, 10.25$ Hz, 2H), 3.51-3.59 (m, 2H), 3.33-3.41 (m, 2H), 2.86 (t, $J=7.25$ Hz, 2H), 2.20-2.28 (m, 2H), 1.88 (quin, $J=6.46$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 172.5, 166.4, 139.6, 131.1, 128.5, 127.6,
15 126.8, 126.1, 40.6, 39.7, 35.4, 33.9, 26.6. HRMS-ESI (m/z): ($\text{M} + \text{H}$) $^+$ calculated for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_2$, 260.1525, obs. 260.3315.

N-Phenethyl-4-(2-nitro-benzenesulfonylamino)butanamide (III-20). Converted to the N-phenylacetamide derivative using the general procedure described and with the
20 following alteration: recrystallization from ethyl acetate and was obtained as a light yellow solid (0.18 g, 77%); mp 78-79 °C. NMR (500 MHz, CDCl_3) δ 8.44-8.49 (m, 1H), 8.07-8.13 (m, 1H), 7.71-7.87 (m, 7H), 7.27-7.35 (m, 3H), 7.17-7.26 (m, 4H), 4.08 (t, $J=6.94$ Hz, 2H), 3.46-3.58 (m, 3H), 3.13 (q, $J=6.52$ Hz, 3H), 2.82 (t, $J=7.09$ Hz, 3H), 2.54 (t, $J=8.04$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 172.1, 148.1, 138.8, 135.0, 134.4, 133.6, 132.8, 132.0, 131.0,
25 128.7, 128.6, 126.5, 125.3, 124.3, 43.1, 40.7, 33.1, 32.1, 25.3, 19.0. HRMS-ESI (m/z): ($\text{M} + \text{H}$) $^+$ calculated for $\text{C}_{18}\text{H}_{22}\text{N}_3\text{O}_5\text{S}$, 392.1280, obs. 392.1282.

N-Phenethyl-4-(tosylamino)butanamide (III-21). Was obtained as a white solid (0.40 g, 81%); mp 126.6 - 127.3 °C. ^1H NMR (500 MHz, CDCl_3) δ 7.72 (d, $J=8.20$ Hz, 2H), 7.26-7.31 (m, 5H), 7.15-7.23 (m, 3H), 5.93 (br. s., 1H), 5.37 (br. s., 1H), 3.42-3.53 (m, 2H),
30 2.93 (q, $J=6.20$ Hz, 2H), 2.79 (t, $J=7.25$ Hz, 2H), 2.21 (t, $J=6.78$ Hz, 2H), 1.81 (s, 2H), 1.77 (quin, $J=6.62$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3) δ 173.4, 145.2, 135.2, 129.7, 128.7, 128.6, 128.0, 127.0, 126.4, 42.6, 40.7, 35.6, 33.4, 32.2, 25.2, 21.7, 18.2. HRMS-ESI (m/z): ($\text{M} + \text{H}$) $^+$ calculated for $\text{C}_{19}\text{H}_{25}\text{SN}_2\text{O}_3$, 361.1586, obs. 361.1580.

S-(3-Oxo-3-((4-oxo-4-(phenethylamino)butyl)amino)propyl)ethanethioate (III-23). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.58 g, 90%); mp 100-101 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.38 (m, 2H), 7.18-7.28 (m, 2H), 6.14 (br. s., 1H), 5.95 (br. s., 1H), 3.47-3.62 (m, 2H), 3.29 (td, *J*=6.03, 12.22 Hz, 2H), 3.16 (t, *J*=6.94 Hz, 1H), 2.84-2.89 (m, 2H), 2.45-2.53 (m, 2H), 2.34 (s, 3H), 2.15-2.24 (m, 2H), 1.78-1.86 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 195.7, 172.6, 170.9, 138.8, 128.7, 128.6, 126.5, 40.6, 39.1, 36.4, 35.6, 33.9, 30.4, 25.4, 20.4. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₇H₂₅N₂O₃S, 337.1586, obs. 337.1583.

4-(3-Mercaptopropanamido)-N-phenethylbutanamide (III-24). S-(3-oxo-3-((4-oxo-4-(phenethylamino)butyl)amino)propyl)ethanethioate (20.0 mg, 0.06 mmol) was dissolved in MeOH (1.0 mL) and acetyl chloride (2 μL, 50 mol %) was added. The reaction was stirred for 2 hrs, additional acetyl chloride (1 μL) was added and the solution was stirred for another 2 hrs. Methylene chloride and H₂O (5 mL each) was added followed by a sat. NaHCO₃ until the reaction attained a pH of 7. The aqueous layer was extracted with methyl chloride (3 x 10 mL) and the organic layers combined and dried over Na₂SO₄. Concentration in vacuo yielded a white solid (14.2 mg, 79%); mp 136 – 137.5 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.29-7.36 (m, 1H), 7.18-7.27 (m, 2H), 6.24 (br. s., 1H), 5.93 (br. s., 1H), 3.52-3.59 (m, 2H), 3.25-3.33 (m, 2H), 3.16 (t, *J*=7.09 Hz, 1H), 2.81-2.89 (m, 3H), 2.46-2.52 (m, 2H), 2.16-2.25 (m, 2H), 1.80-1.87 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 172.6, 171.0, 138.8, 128.7, 128.6, 126.5, 40.6, 40.4, 39.2, 35.6, 34.0, 25.3, 25.0, 20.4. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₅H₂₂N₂O₂S, 295.1480, obs. 295.1501.

N-Phenethyl -4-(2-oxo-pyrrolidine-1-thiocarbonylamino)butanamide (III-38)
Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from dichloromethane / hexane and was obtained as a white solid (0.08 g, 54%); mp 98-99 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 2H), 7.19-7.28 (m, 3H), 5.71 (br. s., 1H), 4.23 (t, *J*=7.09 Hz, 2H), 3.66-3.72 (m, 2H), 3.51-3.59 (m, 2H), 2.85 (t, *J*=7.09 Hz, 2H), 2.72 (t, *J*=8.20 Hz, 2H), 2.23 (t, *J*=7.25 Hz, 2H), 1.96-2.10 (m, 4H). ¹³C NMR (126 MHz, CDCl₃) δ 180.8, 176.7, 171.8, 138.8, 128.8, 128.7, 126.5, 51.1, 44.6, 40.6, 35.7, 34.5, 33.7, 24.2, 16.9. HRMS-ESI (*m/z*): (M + H)⁺ calculated for C₁₇H₂₄N₃O₂S, 334.1589, obs. 334.1604.

N-Phenethyl -4-(2-oxo-pyrrolidine-1-carbonylamino)butanamide (III-39).
Converted to the N-phenylacetamide derivative using the general procedure described and

with the following alteration: recrystallization from dichloromethane / hexane and was obtained as a white solid (0.05 g, 43%) Mp = 68-69 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.48 (br. s., 1H), 7.16-7.43 (m, 5H), 6.03 (br. s., 1H), 3.86 (t, *J*=7.09 Hz, 2H), 3.55 (q, *J*=6.73 Hz, 2H), 3.31 (q, *J*=6.41 Hz, 2H), 2.85 (t, *J*=7.09 Hz, 2H), 2.62 (t, *J*=8.20 Hz, 2H), 2.19 (t, *J*=7.25 Hz, 2H), 2.00-2.11 (m, 2H), 1.82-1.95 (m, 2H). ¹³C NMR (126 MHz, CDCl₃) δ 177.1, 172.2, 153.4, 139.0, 128.8, 128.6, 126.4, 45.7, 40.6, 38.9, 35.7, 33.7, 33.4, 26.2, 17.0. HRMS-ESI (*m/z*): (*M* + *H*)⁺ calculated for C₁₇H₂₄N₃O₃, 318.1818, obs. 318.1823.

Methyl 4-hydroxybutanoate (III-19). γ-Butyrolactone (0.76 mL, 10.0 mmol) was added to methanol (50 mL) and triethylamine (8.40 mL, 60.0 mmol) and the resulting solution was heated at 60 °C for 16 hrs. The solution was then cooled and concentrated to reveal a clear oil and the remaining TEA was removed via azeotrope with hexane (3 x 20 mL). The resulting oil was put under high-vac overnight to yield methyl 4-hydroxybutanoate, as a light amber oil (1.10 g, 9.2 mmol, 92%).

Methyl 4-((ethylcarbamoyl)oxy)butanoate (III-20). Methyl 4-hydroxybutanoate (0.66 g, 5.57 mmol) was dissolved in acetonitrile (26 mL) and to this was added triethylamine (1.6 mL, 11.4 mmol). *N,N'*-disuccinimidyl carbonate (2.20 g, 8.6 mmol) was added portionwise and the solution was stirred at room temperature for 10 hrs. The solution was diluted with ethyl acetate (30 mL) and washed sequential with the following: 0.5 M HCl, H₂O, 50% NaHCO₃, H₂O, and brine (1 x 10 mL) then dried over Na₂SO₄ and concentrate *in vacuo*. The resulting yellow oil was used in the next step without further purification. The crude product was dissolved in THF (5 mL) and triethylamine (0.54 mL, 3.86 mmol). To this was added 68% ethylamine / H₂O solution (10mL) and the reaction was stirred at room temperature for 48 hrs. The reaction mixture was quenched with ammonia chloride (20 mL), diluted with ethyl acetate (30 mL) and washed with brine (2 x 20mL). The solution was dried over Na₂SO₄ and concentrated *in vacuo*. The crude oil was purified by flash column chromatography (20% ethyl acetate / hexane) to yield methyl 4-((ethylcarbamoyl)oxy)butanoate as a clear oil (0.25 g, 1.35 mmol, 35%).

4-Oxo-4-(phenethylamino)butyl ethylcarbamate, (III-21). Methyl 4-((ethylcarbamoyl)oxy)butanoate (0.25 g, 1.35 mmol) was dissolved in THF (3 mL) and to this was added aqueous LiOH (0.25 g, 6.10 mmol, 1M) at room temperature and allowed to stir overnight. The reaction mixture was acidified to pH = 3 by litmus paper with 1M HCl (40 mL). The aqueous layer was extracted with ethyl acetate (3 x 50 mL), dried over Na₂SO₄,

and concentrated *in vacuo*. The resulting solid was recrystallized from ethyl acetate:hexane to yield a white solid which was in the next reaction without further purification.

The solid was then converted to the N-phenylacetamide derivative using the general procedure described and was obtained as a white solid (0.21, 0.76 mmol, 56% over two steps); mp 110-111 °C.

Butyl (4-oxo-4-(phenethylamino)butyl)carbamate (III-5). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.58 g, 90%) mp 88-89 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.38 (m, 2H), 7.19-7.27 (m, 3H), 6.05 (br. s., 1H), 5.00 (br. s., 1H), 4.05 (t, *J*=6.66 Hz, 2H), 3.51-3.56 (m, 2H), 3.19 (q, *J*=6.28 Hz, 2H), 2.84 (t, *J*=7.09 Hz, 2H), 2.19 (t, *J*=7.01 Hz, 2H), 1.81 (quin, *J*=6.78 Hz, 2H), 1.59 (quin, *J*=7.05 Hz, 2H), 1.38 (qd, *J*=7.43, 15.00 Hz, 2H), 0.94 (t, *J*=7.41 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 172.7, 157.5, 139.1, 128.9, 128.8, 126.7, 64.9, 40.8, 40.3, 35.8, 33.9, 31.3, 26.4, 19.3, 13.9 HRMS-ESI (*m/z*): (M + H)⁺ calcd for C₁₇H₂₆N₂O₃, 307.2022, obs. 307.2044.

Propyl (4-oxo-4-(phenethylamino)butyl)carbamate (III-4). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.71 g, 95 %) mp 98-99 °C. ¹H NMR (500 MHz, CDCl₃) δ 7.30-7.36 (m, 2H), 7.19-7.27 (m, 3H), 6.09 (br. s., 1H), 5.04 (br. s., 1H), 4.01 (t, *J*=6.74 Hz, 2H), 3.49-3.57 (m, 2H), 3.19 (q, *J*=6.31 Hz, 2H), 2.84 (t, *J*=7.09 Hz, 2H), 2.19 (t, *J*=7.01 Hz, 2H), 1.81 (quin, *J*=6.78 Hz, 2H), 1.63 (sxt, *J*=7.06 Hz, 2H), 0.94 (t, *J*=7.41 Hz, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 172.7, 157.5, 139.1, 128.9, 128.8, 126.7, 66.7, 40.8, 40.3, 35.8, 33.9, 26.4, 22.5, 10.5 HRMS-ESI (*m/z*): (M + H)⁺ calcd for C₁₆H₂₄N₂O₃, 293.1865, obs. 293.1861.

Prop-2-yn-1-yl (4-oxo-4-(phenethylamino)butyl)carbamate (III-7). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.58 g, 90%) mp 86 °C. ¹H NMR (500 MHz, CHLOROFORM-*d*) δ 7.30-7.35 (m, 2H), 7.19-7.27 (m, 3H), 5.95 (br. s., 1H), 5.23-5.36 (m, 1H), 4.67 (d, *J*=2.29 Hz, 2H), 3.50-3.57 (m, 2H), 3.22 (q, *J*=6.31 Hz, 2H), 2.84 (t, *J*=7.01 Hz, 2H), 2.20 (t, *J*=6.98 Hz, 2H), 1.83 (quin, *J*=6.78 Hz, 2H). ¹³C NMR (126 MHz, CHLOROFORM-*d*) δ 172.6, 156.1, 139.0, 128.9, 128.8, 78.5, 74.7, 52.6, 40.8, 40.6, 35.8, 33.8, 26.0. HRMS-ESI (*m/z*): (M + H)⁺ calcd for C₁₆H₂₀N₂O₃, 289.1552, obs. 289.1578.

2-Fluoroethyl (4-oxo-4-(phenethylamino)butyl)carbamate (III-9). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.68 g, 81 %) mp 95 °C. ¹H NMR (500 MHz, CHLOROFORM-d) δ 7.31-7.37 (m, 2H), 7.19-7.28 (m, 3H), 5.86 (br. s., 1H), 5.17 (br. s., 1H), 4.62-4.68 (m, 1H), 4.51-4.58 (m, 1H), 4.32-4.38 (m, 1H), 4.26-4.31 (m, 1H), 3.52-3.59 (m, 2H), 3.22 (q, *J*=6.38 Hz, 2H), 2.85 (t, *J*=7.01 Hz, 2H), 2.20 (t, *J*=7.01 Hz, 2H), 1.84 (quin, *J*=6.80 Hz, 2H). ¹³C NMR (126 MHz, CHLOROFORM-d) δ 172.6, 156.6, 139.0, 128.9, 128.8, 126.7, 82.7, 81.4, 64.1, 63.9, 40.8, 40.5, 35.8, 33.8, 26.0. HRMS-ESI (*m/z*): (M + H)⁺ calcd for C₁₅H₂₁N₂O₃F, 297.1631, obs. 297.1638.

Chloromethyl (4-oxo-4-(phenethylamino)butyl)carbamate (III-8). Converted to the N-phenylacetamide derivative using the general procedure described and with the following alteration: recrystallization from warm petroleum ether and was obtained as a white solid (0.58 g, 90%) mp 92-93 °C. ¹H NMR (500 MHz, METHANOL-d₄) δ 7.24-7.34 (m, 2H), 7.13-7.24 (m, 3H), 3.40 (t, *J*=7.37 Hz, 2H), 3.35 (t, *J*=7.21 Hz, 2H), 3.29-3.33 (m, 2H), 3.08 (t, *J*=6.94 Hz, 2H), 2.78 (td, *J*=7.25, 14.19 Hz, 4H), 2.12-2.20 (m, 2H), 1.70 (quin, *J*=7.21 Hz, 2H). ¹³C NMR (126 MHz, METHANOL-d₄) δ 172.9, 158.4, 138.1, 137.8, 127.1, 126.7, 124.6, 124.5, 39.9, 39.2, 37.7, 34.8, 33.8, 31.6, 25.0. HRMS-ESI (*m/z*): (M + H)⁺ calcd for C₁₄H₁₉N₂O₃Cl, 298.1100, obs. 298.1105.

Biological Evaluation. HDAC Enzyme Inhibition Assay. Determination of IC₅₀ values was performed using Class I HDAC (HDAC-2) and Class II HDAC (HDAC6), commercially available human recombinant enzymes (BPS Bioscience, San Diego, CA) and a fluorogenic HDAC assay kit (Active Motif, Carlsbad CA for HDAC2 and BPS Bioscience, San Diego, CA for HDAC6). VORINOSTAT (SAHA; Sigma Aldrich, St. Louis, MO) served as a control for the enzyme inhibition assay. Assay data was subjected to non-linear regression analysis (GraphPad Software, Inc., CA). IC₅₀ enzyme inhibition assays were performed with varying concentrations on all completed synthetic analogues. Components were added sequentially to a black, flat bottom 96-well assay plate (Sigma-Aldrich) as described by the manufacturer's protocol and the reaction mixture was incubated for thirty minutes at 37 °C. Trichostatin A, included in the assay kit at a final reaction concentration of 1 μM was added to the bifunctional HDAC assay developer to stop deacetylation and initiate fluorescent signal releasing fluorophore. The reaction mixture was further incubated at room temperature for fifteen minutes. Fluorescence was measured on a Spectra Max Gemini XPS

(Molecular Devices, Sunnyvale CA) using an excitation wavelength of 360 nm and a detection wavelength of 460 nm. IC₅₀ values were determined in nanomolar concentrations via subjection to non-linear regression analysis (GraphPad Software, Inc., CA).

Cellular Anti-Proliferation Assay. Human colon cancer cells (HCT-116) were
5 obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultivated according to supplier's instructions using McCoy's 5A media supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% non-essential amino acids. Upon subconfluent growth, cells were seeded in a 96-well plate at 5,000 cells per well. Before
10 treatment, the plates were incubated at 37 °C, 5% CO₂ for 24 hours. Treatment with test compounds was carried out in triplicate wells for 96 hours using SAHA as a positive control.

Human cutaneous T lymphocyte (HuT-78) cells were obtained from ATCC and cultivated according to supplier's instructions using Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. Cells were seeded at log growth phase at 50,000 cells per well. Plates were incubated at
15 37 °C, 5% CO₂ for 4 hours, treated with compounds in triplicate, and incubated for 72 hours using SAHA as a positive control.

Human dermal fibroblast cells (hDF) were obtained from ATCC and cultivated according to supplier's instructions using Dulbecco's Modified Eagle Medium (DMEM) F-12 media supplemented with 40% FBS and 1% penicillin/streptomycin. Cells were seeded at
20 5,000 cells per well and incubated at 37 °C, 5% CO₂ for 24 hours (adherent cells). Treatment with compounds was carried out in triplicate for 72 hours using SAHA as a positive control. GI₅₀ cell cytotoxicity values were determined by varying concentrations of all compounds and assay data was subjected to non-linear regression analysis (GraphPad Software, Inc.,
25 CA).

RESULTS

Biological Evaluation. Initial biological assessment involved evaluation in a fluorogenic Class I HDAC assay (Active Motif) for their IC₅₀ values in recombinant HDAC2 (BPS Bioscience, San Diego, CA), Class II HDAC6 assay (BPS Bioscience, San Diego, CA),
30 and antiproliferative activity screening at 50 µM and subsequent GI₅₀ values in HCT-116 and HuT-78 cells by standard 3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reduction assay (Promega). Initial screening in the enzyme inhibition assay was performed at 1000 and 500 nM with all compounds displaying

inhibition values greater than 50% at 500nM investigated further and IC₅₀ values obtained and 50 and 10 μ M screening for the cell assay with all compounds displaying cell growth inhibition greater than 50% at 50 μ M investigated further and GI₅₀ values obtained (ZBG-group analogues summarized in Table 3 and Table 4). HDAC proteins consist of at least 18 enzymes divided into four groups: Class I (1, 2, 3, and 8) Class IIa (4, 5, 7, and 9), Class IIb (6 and 10), Class III, and Class IV (11). Class I is ubiquitously expressed in all cells and regarded as showing the most activity in tumor regulation and corepression as such selectivity and potency within Class I is ideal. Moreover the HCT-116 (solid tumor) and HuT-78 cells (lymphoma) are cancerous cell lines, with known susceptibility to some HDAC inhibitors, and are well-suited to test the cell potency of potential HDAC inhibitors. The specific HDACs overexpressed in either cell line have not been examined or quantified. Furthermore, all derivatives were screened in human dermal fibroblast (hDF) cells at 100 μ M to test for non-specific cytotoxicity. See Tables 3 and 4.

Table 3. HDAC inhibition and Cytotoxic Activity Analogs III-1 to III-18, III-21, III-22

	HDAC2	HDAC6		HCT116	HuT-78	hDF
	IC ₅₀	IC ₅₀	Selectivity Index	GI ₅₀	GI ₅₀	GI ₅₀
Compd	(nM)	(nM)	(HDAC6:HDAC2)	(μ M)	(μ M)	(μ M)
SCA	0.111	395.0	3347	29.0	1.31	>100
SCA-SAHA	2.35	328.4	140.0	1.92	0.66	>100
SAHA	85.8	38.9	0.45	0.4	3.0	6.1
III-1	0.115	395.1	3435.7	27.8	1.34	>100
III-2	0.257	>500	>1945.5	>50	3.1	>100
III-3	479.0	>500	>1.04	>50	24.8	>100
III-4	0.14	>500	>3448.3	>50	2.36	>100
III-5	0.18	>500	>2645.5	49.3	0.86	>100
III-6	28.2	64.6	2.30	32.4	>50	>100
III-7	0.38	>500	>1288.7	>50	7.64	>100
III-8	3.57	>500	>139.9	>50	1.94	>100
III-9	1.94	>500	>257.7	>50	1.12	>100
III-10	48.8	>500	>10.2	>50	>50	>100
III-11	N/A	N/A	N/A	N/A	N/A	N/A
III-12	1.50	>500	>333.3	19.4	2.75	>100
III-13	>500	>500	~1.0	>50	26.0	>100

III-14	> 500	>500	~1.0	39.8	19.3	>100
III-15	250.0	74.7	0.30	>50	>50	>100
III-16	>500	80.3	<0.16	21.4	7.24	>100
III-17	21.5	>500	>23.3	12.5	10.3	>100
III-18	>500	>500	~1.0	18.5	>50	>100
III-21	1.50	>500	>333.3	19.4	2.75	>100
III-22	3.26	>500	>153.4	12.4	1.81	>100

Table 4. HDAC inhibition and Cytotoxic Activity of Analogs III-23 to III-30, III-32, III-33, III-38, and III-39

Compd	HDAC2	HDAC6	Selectivity Index (HDAC6:HDAC2)	HCT116	HuT-78	hDF
	IC ₅₀ (nM)	IC ₅₀ (nM)		GI ₅₀ (μM)	GI ₅₀ (μM)	GI ₅₀ (μM)
III-23	71.6	>500	>7.0	47.9	5.5	>100
III-24	>500	>500	~1.0	>50	>50	>100
III-25	>500	>500	~1.0	>50	>50	>100
III-26	>500	>500	~1.0	19.4	>50	>100
III-27	368.2	>500	>1.36	36.1	>50	>100
III-28	>500	>500	~1.0	>50	>50	>100
III-29	1.91	79.4	41.6	>50	>50	>100
III-30	500.0	>500	>1.0	18.57	11.4	>100
III-32	11.6	>500	>43.1	0.32	0.62	>100
III-33	1.04	>500	>480.8	0.21	0.36	>100
III-38	>500	>500	~1.0	45.3	>50	>100
III-39	18.5	62.8	3.4	9.41	7.7	>100
III-40	115.26	>500	>4.3	>50	11.6	>100

Example 3: Cap Group Analogs (Formula IV) and Linker Group Analogs (Formula V) of Santacruzamate A

The synthetic methodology for **Formula IV** was the following: starting from γ -aminobutyric acid, conversion to the ethyl carbamate proceeded through standard acylation conditions via reaction with ethyl chloroformate using excess potassium carbonate in water. All cap-derivatives were then coupled to the free acid via previously described EDC coupling procedures. For sluggish coupling reactions and all neurotransmitters (**IV-15** through **IV-17**), PyBOP/HOBt mediated-couplings were utilized. A total of twenty cap-derivatives were synthesized and evaluated for their bioactivity.

Initial biological assessment involved evaluation in a fluorogenic Class I HDAC assay (Active Motif) for their IC₅₀ values in recombinant HDAC2 (BPS Bioscience, San Diego, CA), Class II HDAC6 assay (BPS Bioscience, San Diego, CA), and antiproliferative activity screening at 50 uM and subsequent GI₅₀ values in HCT-116 and HuT-78 cells by standard 3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)

reduction assay (Promega). Initial screening in the enzyme inhibition assay was performed at 500 nM with all compounds displaying inhibition values greater than 50% at 500 nM investigated further and IC₅₀ values obtained. Cellular assays screened at 50 and 10 uM with all compounds displaying cell growth inhibition greater than 50% at 50 uM investigated further and GI₅₀ values obtained (cap-group analogues summarized in Table 5). HDAC proteins consist of at least 18 enzymes divided into four groups: Class I (1, 2, 3, and 8) Class IIa (4, 5, 7, and 9), Class IIb (6 and 10), Class III, and Class IV (11). Class I is ubiquitously expressed in all cells and regarded as showing the most activity in tumor regulation and co-repression as such selectivity and potency within Class I is ideal. Moreover, the HCT-116 and HuT-78 cells are cancerous cell lines, known for their susceptibility to some HDAC inhibitors, and are well-suited to test the cell potency of potential HDAC inhibitors. The specific HDACs overexpressed in either cell line have not been examined or quantified. Lastly, each derivative was tested for non-specific cytotoxicity in human dermal fibroblast cells (hDF).

Table 5: Bioassay data for cap-modified analogues

Compound	HDAC2 IC ₅₀ (nM)	HDAC6 IC ₅₀ (nM)	Selectivity Index (HDAC6:HDAC2)	HCT116 GI ₅₀ (μM)	HuT-78 GI ₅₀ (μM)	hDF GI ₅₀ (μM)
SCA	0.111	395.0	3347	29.0	1.34	>100
SCA-SAHA	2.35	328.4	140.0	1.92	0.66	>100
SAHA	85.8	38.9	0.45	0.4	3.0	6.1
IV-1	134.1	268.0	1.99	>50	35.6	>100
IV-2	483.1	>500	>1.03	>50	>50	>100
IV-3	1.21	>500	>413.2	>50	17.64	>100
IV-4a	21.4	>500	>23.4	>50	>50	>100
IV-4b	1.43	>500	>349.6	7.67	0.99	>100
IV-4c	3.45	>500	>145.4	>50	5.69	>100
IV-5	6.09	>500	>82.1	>50	18.85	>100
IV-6	1.84	>500	>271.7	>50	6.39	>100
IV-7	483.3	>500	>1.03	>50	>50	>100
IV-8	>500	>500	n/a	>50	15.8	>100
IV-9	1.98	>500	>252.5	>50	4.75	>100
IV-10	2.25	>500	>222.2	>50	>50	>100
IV-11	2.25	222.7	99.0	1.78	0.47	>100

IV-12	21.4	53.1	2.48	>50	>50	>100
IV-13	49.84	>500	>10.0	>50	>50	>100
IV-14	163.3	>500	>3.06	>50	>50	>100
IV-15	72.8	>500	>6.86	>50	38.9	>100
IV-16	6.06	>500	>82.5	>50	>50	>100
IV-17	1.18	>500	>423.7	>50	5.18	>100
IV-18	>500	260.6	< 0.52	>50	50.8	>100

The synthetic strategy for linker modification (**Formula V**) examined both the importance of the 3-carbon length in the SCA-scaffold as well as the relative spatial position of the amide bond which joins the cap group to the aliphatic linker. Phase I involved compression and expansion of the 3-carbon linker connecting the ethyl carbamate ZBG-group to the phenethylamine cap group. The biological data are shown in Table 6.

Table 6: Bioassay Data for Linker-modified Analogues						
	HDAC2	HDAC6	Selectivity Index	HCT116	HuT-78	hDF
Compound	IC ₅₀ (nM)	IC ₅₀ (nM)	(HDAC6:HDAC2)	IC ₅₀ (μM)	IC ₅₀ (μM)	GI ₅₀ (μM)
SCA	0.111	395.0	3347	29.0	1.34	>100
V-19	50.5	>500	>9.9	>50	3.52	>100
V-20	>500	>500	~1.0	>50	18.7	>100
V-21	2.43	>500	>205	>50	10.94	>100
V-22	0.58	>500	>862.1	>50	1.69	>100
V-23	1.29	>500	>387.6	>50	4.12	>100
V-24	175.5	>500	>2.85	>50	24.8	>100
V-25	0.97	>500	>515.5	>50	3.69	>100
V-26	2.8	>500	>178.6	>50	31.3	>100
V-27	1.99	>500	>251.3	>50	>50	>100

In conclusion, 29 new analogs of the potent, selective natural product Santacruzamate A have been developed.

General procedure for formation of cap and linker modified SCA analogs

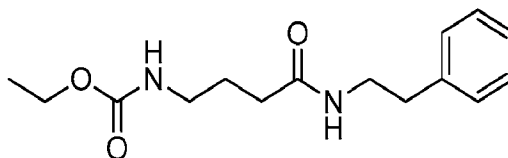
Amino acid (4.85 mmol) was dissolved in H₂O (7 mL). Once dissolved, K₂CO₃ (12.6 mmol) was added and the resulting solution was cooled to 0°C. Ethyl chloroformate (6.31 mmol) was added dropwise and the solution was stirred at 0°C for 2h and then stirred
5 overnight at room temperature. The reaction mixture was then diluted with H₂O (20 mL) and extracted with EtOAc (3 x 10mL). The aqueous phase was acidified to pH = 2 with cold concentrated HCl and extracted with EtOAc (3 x 20 mL). The EtOAc extracts were dried over Na₂SO₄, concentrated to reveal the product which was recrystallized from the appropriate solvent (ethyl acetate/hexane).

10 The subsequent ethyl carbamate intermediate (2.28 mmol) was dissolved in CH₂Cl₂ (7 mL) and cooled to 0 °C. Phenethylamine (2.60 mmol) and triethylamine (4.56 mmol) were added to the solution followed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.50 g, 2.60 mmol) in one portion. 4-Dimethylaminopyridine (cat.) was added and the solution was stirred at 0 °C for 60 minutes then overnight at room temperature.
15 The resulting solution was diluted with additional CH₂Cl₂ (20 mL) and sequentially washed with 10 mL of each of the following: 1.0 M HCl, H₂O, sat. NaHCO₃, H₂O, brine. The organic layer was dried over Na₂SO₄ and concentrated to give a residue which was pushed through a plug of silica (100% ethyl acetate), titrated with hexane then recrystallized from ethyl acetate/hexane. Upon cooling to 0 °C, the solution was filtered to yield pure product.

20 The compounds and methods of the appended claims are not limited in scope by the specific compounds and methods described herein, which are intended as illustrations of a few aspects of the claims and any compounds and methods that are functionally equivalent are within the scope of this disclosure. Various modifications of the compounds and methods in addition to those shown and described herein are intended to fall within the scope of the
25 appended claims. Further, while only certain representative compounds, methods, and aspects of these compounds and methods are specifically described, other compounds and methods and combinations of various features of the compounds and methods are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents may be explicitly mentioned
30 herein; however, all other combinations of steps, elements, components, and constituents are included, even though not explicitly stated. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entireties for all purposes.

WHAT IS CLAIMED IS:

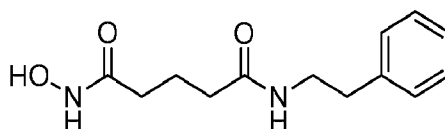
1. A compound having the following structure:



or a pharmaceutically acceptable salt or prodrug thereof.

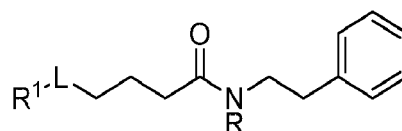
2. The compound of claim 1, wherein the compound is a synthetic compound.

3. A compound having the following structure:

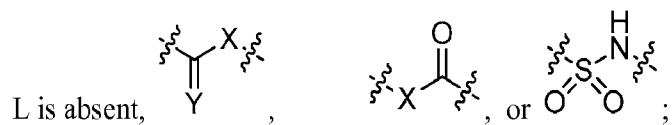


or a pharmaceutically acceptable salt or prodrug thereof.

4. A compound having the following structure:



or a pharmaceutically acceptable salt or prodrug thereof, wherein:



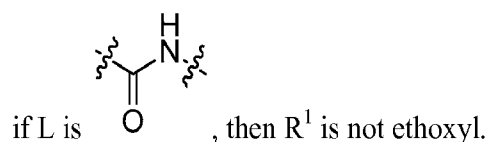
X is NR or O;

Y is O or S;

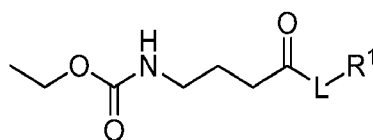
each R is independently selected from hydrogen or substituted or unsubstituted C₁₋₆ alkyl; and

R¹ is hydrogen, alkoxy, aryloxy, substituted or unsubstituted amino, substituted or unsubstituted thiol, substituted or unsubstituted C₁₋₆ alkyl, substituted or unsubstituted C₂₋₆ alkenyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted cycloalkyl, or substituted or unsubstituted heterocyclyl,

wherein if L is absent, then R¹ is substituted or unsubstituted amino; and



5. A compound having the following structure:

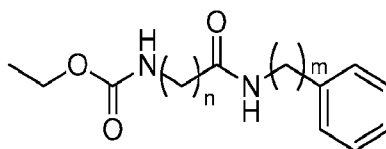


or a pharmaceutically acceptable salt or prodrug thereof, wherein:

L is NH, O, S, or CH₂; and

R¹ is substituted or unsubstituted C₁₋₆ alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl,
wherein if L is NH, then R¹ is not -CH₂CH₂Ph.

6. A compound having the following structure:



or a pharmaceutically acceptable salt or prodrug thereof, wherein:

m is 0 to 5; and

n is 1 to 5,

wherein if m is 2, then n is not 3.

7. The compound of claim 6, wherein m is 2.
8. A method of inhibiting histone deacetylase (HDAC) activity in a cell, comprising contacting an HDAC isoform with a compound of any one of claims 1-7.
9. The method of claim 8, wherein the HDAC isoform is HDAC-2.
10. The method of claim 8, wherein the HDAC isoform is HDAC-6.
11. The method of any one of claims 8-10, wherein the contacting is performed *in vivo*.
12. The method of any one of claims 8-10, wherein the contacting is performed *in vitro*.
13. A method of inhibiting HDAC activity in a subject, comprising administering to the subject a compound of any one of claims 1-7.
14. The method of claim 13, wherein the HDAC activity is HDAC-2 activity.

15. The method of claim 13, wherein the HDAC activity is HDAC-6 activity.
16. A method of treating cancer in a subject, comprising administering to the subject an effective amount of a compound of any one of claims 1-7.
17. The method of claim 16, wherein the cancer is breast cancer or colon cancer.
18. The method of claim 16, wherein the cancer is cutaneous T-cell lymphoma.
19. The method of any of claims 16-18, further comprising administering a therapeutic agent to the subject.
20. The method of claim 19, wherein the therapeutic agent is a chemotherapeutic agent.
21. A method of treating a neurological disorder in a subject, comprising administering to the subject an effective amount of a compound of any one of claims 1-7.
22. The method of claim 21, further comprising administering a therapeutic agent to the subject.
23. The method of claim 22, wherein the therapeutic agent is an anti-depressant or an anxiolytic.
24. A pharmaceutical formulation comprising a compound of any one of claims 1-7 and a pharmaceutically acceptable carrier.
25. The pharmaceutical formulation of claim 24, wherein the pharmaceutical formulation is a solid pharmaceutical formulation.
26. The pharmaceutical formulation of claim 24, wherein the pharmaceutical formulation is a liquid pharmaceutical formulation and the pharmaceutically acceptable carrier is an aqueous medium.