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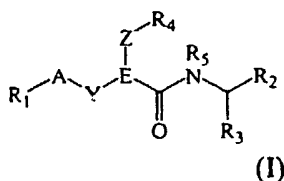
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(54) Title: INHIBITORS OF BACTERIAL TYPE III PROTEIN SECRETION SYSTEMS



(57) Abstract: In accordance with the present invention, compounds having formula (I), where the variables are as identified in the claims, that inhibit Type III protein secretion have been identified, and methods for their use provided. In one aspect of the invention, compounds useful in the inhibition of Type III protein secretion and/or in the treatment and prevention of bacterial infections, particular Gram-negative bacterial infections, are provided. In another aspect of the invention, methods are provided for the inhibition of Type III protein secretion and/or the treatment and prevention of bacterial infections, particular Gram-negative bacterial infections using the compounds of the invention.

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INHIBITORS OF BACTERIAL TYPE III PROTEIN SECRETION SYSTEMS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. 119(e) of provisional application, Serial Number 60/568,854, filed May 7, 2004.

FIELD OF THE INVENTION

The subject invention relates to novel anti-microbial compounds, their compositions and their uses.

BACKGROUND OF THE INVENTION

Type III protein secretion systems are an essential virulence determinant of most pathogenic Gram-negative bacteria, including *Salmonella*, *Shigella*, *Yersinia*, *Pseudomonas aeruginosa*, and enteropathogenic *Escherichia coli*. The Type III virulence mechanism consists of a secretion apparatus, consisting of about 25 proteins, and a set of effector proteins released by this apparatus. Following activation by intimate contact with a eukaryotic cell membrane, the effector proteins are injected into the host cell, where they subvert the signal transduction machinery and lead to a variety of host cell responses. This virulence mechanism plays a key role in establishing and maintaining an infection and in the resulting pathophysiological sequelae, such as diarrhea, chronic lung inflammation, and septicemia.

Certain protein components of the Type III secretion apparatus are highly conserved among bacterial pathogens, and as such represent suitable targets for therapeutic intervention. Inhibitors of Type III protein secretion are expected to be useful as prophylactic agents (i.e., to prevent the onset of infection by Gram-negative bacteria) or as drugs to treat an existing bacterial infection, either with or without an anti-bacterial agent.

There remains a need to develop, characterize, and optimize lead molecules for the development of novel anti-bacterial drugs. Accordingly, it is an object of the present invention to provide such compounds.

SUMMARY OF THE INVENTION

In accordance with the present invention, compounds that inhibit Type III protein secretion have been identified, and methods for their use provided.

In one aspect of the invention, compounds of Formula (I) are provided which are useful in the inhibition of Type III protein secretion and/or in the treatment and prevention of bacterial infection, particularly Gram-negative bacterial infection.

In another aspect of the invention, methods are provided for the inhibition of Type III protein secretion and/or in the treatment and prevention of bacterial infection, particularly Gram-negative bacterial infection using the compounds described herein.

In one embodiment, the invention is directed to methods for inhibiting Type III protein secretion comprising administering a secretion-inhibiting amount of at least one compound of the invention to a subject in need thereof.

In another embodiment, methods for treating and/or preventing bacterial infection, particularly Gram-negative bacterial infection, are provided comprising administering a therapeutically or prophylactically effective amount of at least one compound of the invention to a subject in need thereof.

These and other aspects of the invention will be more clearly understood with reference to the following preferred embodiments and detailed description.

DETAILED DESCRIPTION OF THE INVENTION

Inhibition of Type III protein secretion is an important factor in the treatment and prevention of infection by Gram-negative bacteria. In accordance with the present invention, compounds that inhibit Type III protein secretion have been identified, and methods for their use provided.

A. Compounds of the Invention

In one aspect of the invention, compounds of the invention are provided which are useful in the inhibition of bacterial Type III protein secretion systems, and/or in the treatment or prevention of bacterial infection, particularly Gram-negative bacterial infection.

Where the compounds according to this invention have at least one stereogenic center, they may accordingly exist as enantiomers. Where the compounds possess two or more stereogenic centers, they may additionally exist as diastereomers. Furthermore, some of the crystalline forms for the compounds may exist as polymorphs and as such are intended to be included in the present invention. In addition, some of the compounds may form solvates with water (i.e., hydrates) or common organic solvents, and such solvates are also intended to be encompassed within the scope of this invention.

Some of the compounds of the present invention may have trans and cis isomers. In addition, where the processes for the preparation of the compounds according to the invention give rise to a mixture of stereoisomers, these isomers may be separated by conventional techniques such as preparative chromatography. The compounds may be prepared as a single stereoisomer or in racemic form as a mixture of some possible stereoisomers. The non-racemic forms may be obtained by either synthesis or resolution. The compounds may, for example, be resolved into their component enantiomers by standard techniques, such as the formation of diastereomeric pairs by salt formation. The compounds may also be resolved by covalent linkage to a chiral auxiliary, followed by chromatographic separation and/or crystallographic separation, and removal of the chiral auxiliary. Alternatively, the compounds may be resolved using chiral chromatography.

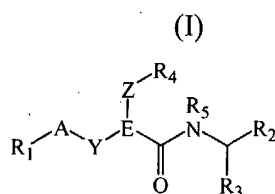
Certain of the compounds of the invention, for example the imidazole derivatives, may exist as tautomers. It is understood that such tautomeric forms are intended to be encompassed within the scope of the invention.

As used herein, "enantiomerically pure" refers to compositions consisting substantially of a single isomer, preferably consisting of 90%, 92%, 95%, 98%, 99%, or 100% of a single isomer.

Included within the scope of the invention are the hydrated forms of the compounds that contain various amounts of water, for instance, the hydrate, hemihydrate,

and sesquihydrate forms. The present invention also includes within its scope prodrugs and pharmaceutically acceptable salts of the compounds of this invention. In general, such prodrugs will be functional derivatives of the compounds that are readily convertible *in vivo* into the required compound. Thus, in the methods of treatment of the present invention, the term “administering” shall encompass the treatment of the various disorders described with the compound specifically disclosed or with a compound which may not be specifically disclosed, but which converts to the specified compound *in vivo* after administration to the patient. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in “Design of Prodrugs”, ed. H. Bundgaard, Elsevier, 1985.

Preferred compounds of the present invention useful in the inhibition of Type III protein secretion include those of Formula (I) as shown below.



wherein A is -C(O)- or -CH₂-;

Y is -NH- or -CH₂-;

E-Z is -C=CH- or -N-CH₂-;

R₁ is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, or substituted heteroaryl;

R₂ is hydrogen, carboxy, carboxymethyl, or hydroxymethyl;

R₃ is hydrogen, heterocyclyl, substituted heterocyclyl, heteroaryl, substituted heteroaryl, lower alkyl, substituted lower alkyl, aryl, or substituted aryl;

R₄ is aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, or substituted heterocyclyl;

R₅ is hydrogen or lower alkyl;

R₂ and R₃ can combine to form a C₄-C₈ cycloalkyl, optionally substituted by carboxy;

R₃ and R₅ can combine to form a heterocycle, optionally substituted at one to three positions thereof or an optical isomer, diastereomer or enantiomer thereof; or a pharmaceutically acceptable salt, hydrate, or prodrug thereof.

Relative to the above description, certain definitions apply as follows.

Unless otherwise noted, under standard nomenclature used throughout this disclosure the terminal portion of the designated side chain is described first, followed by the adjacent functionality toward the point of attachment.

Unless specified otherwise, the terms "alkyl," "alkenyl," and "alkynyl," whether used alone or as part of a substituent group, include straight and branched chains having 1 to 8 carbon atoms, or any number within this range. The term "alkyl" refers to straight or branched chain hydrocarbons. "Alkenyl" refers to a straight or branched chain hydrocarbon with at least one carbon-carbon double bond. "Alkynyl" refers to a straight or branched chain hydrocarbon with at least one carbon-carbon triple bond. For example, alkyl radicals include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl, 3-(2-methyl)butyl, 2-pentyl, 2-methylbutyl, neopentyl, n-hexyl, 2-hexyl and 2-methylpentyl.

"Alkoxy" radicals are oxygen ethers formed from the previously described straight or branched chain alkyl groups.

"Cycloalkyl" groups contain 3 to 8 ring carbons and preferably 5 to 7 ring carbons.

The alkyl, alkenyl, alkynyl, cycloalkyl groups and alkoxy groups may be independently substituted with one or more members of the group including, but not limited to, halogen, alkyl, alkenyl, alkynyl, cycloalkyl, oxo, aryl, heteroaryl, heterocyclo, CN, nitro, -OCOR₅, -OR₅, -SR₅, -SOR₅, -SO₂R₅, -COOR₅, -NR₅R₆, -CONR₅R₆, -OCONR₅R₆, -NHCOR₅, -NHCOOR₅, -NHC(NH)NHNO₂, and -NHCONR₅R₆, wherein R₅ and R₆ are independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclo, aralkyl, heteroaralkyl, and heterocycloalkyl, or alternatively R₅ and R₆ may join to form a heterocyclic ring containing the nitrogen atom to which they are attached.

The term "acyl" as used herein, whether used alone or as part of a substituent group, means an organic radical having 2 to 6 carbon atoms (branched or straight chain) derived from an organic acid by removal of the hydroxyl group. The term "Ac" as used herein, whether used alone or as part of a substituent group, means acetyl.

The term "halo" or "halogen" means fluoro, chloro, bromo or iodo. (Mono-, di-, tri-, and per-)halo-alkyl is an alkyl radical substituted by independent replacement of the hydrogen atoms thereon with halogen.

"Aryl" or "Ar," whether used alone or as part of a substituent group, is a carbocyclic aromatic radical including, but not limited to, phenyl, 1- or 2- naphthyl and the like. The carbocyclic aromatic radical may be substituted by independent replacement of 1 to 3 of the hydrogen atoms thereon with aryl, heteroaryl, halogen, OH, CN, mercapto, nitro, amino, C₁-C₈-alkyl, C₂-C₈-alkenyl, C₁-C₈-alkoxy, C₁-C₈-alkylthio, C₁-C₈-alkyl-amino, di (C₁-C₈-alkyl)amino, (mono-, di-, tri-, and per-) halo-alkyl, formyl, carboxy, alkoxycarbonyl, C₁-C₈-alkyl-CO-O-, C₁-C₈-alkyl-CO-NH-, or carboxamide. Illustrative aryl radicals include, for example, phenyl, naphthyl, biphenyl, fluorophenyl, difluorophenyl, benzyl, benzoyloxyphenyl, carboethoxyphenyl, acetylphenyl, ethoxyphenyl, phenoxyphenyl, hydroxyphenyl, carboxyphenyl, trifluoromethylphenyl, methoxyethylphenyl, acetamidophenyl, tolyl, xylyl, dimethylcarbamylphenyl and the like. "Ph" or "PH" denotes phenyl. "Bz" denotes benzoyl.

Whether used alone or as part of a substituent group, "heteroaryl" refers to a cyclic, fully unsaturated radical having from five to ten ring atoms of which one ring atom is selected from S, O, and N; 0-2 ring atoms are additional heteroatoms independently selected from S, O, and N; and the remaining ring atoms are carbon. The radical may be joined to the rest of the molecule via any of the ring atoms. Exemplary heteroaryl groups include, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isoxazolyl, thiadiazolyl, triazolyl, triazinyl, oxadiazolyl, thienyl, furanyl, quinolinyl, isoquinolinyl, indolyl, isothiazolyl, N-oxo-pyridyl, 1,1-dioxothienyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl-N-oxide, benzimidazolyl, benzisothiazolyl, benzisoxazolyl, benzodiazinyl, benzofurazanyl, indazolyl, indoliziny, benzofuryl, cinnolinyl, quinoxaliny, pyrrolopyridinyl, furopyridinyl (such as furo[2,3-c]pyridinyl, furo[3,2-b]pyridinyl, or furo[2,3-b]pyridinyl),

imidazo-pyridinyl (such as imidazo[4,5-b]pyridinyl or imidazo[4,5-c]pyridinyl), naphthyridinyl, phthalazinyl, purinyl, pyridopyridyl, quinazolinyl, thienofuryl, thienopyridyl, and thienothienyl. The heteroaryl group may be substituted by independent replacement of 1 to 3 of the hydrogen atoms thereon with aryl, heteroaryl, halogen, OH, CN, mercapto, nitro, amino, C₁-C₈-alkyl, C₁-C₈-alkoxyl, C₁-C₈-alkylthio, C₁-C₈-alkyl-amino, di(C₁-C₈-alkyl)amino, (mono-, di-, tri-, and per-) halo-alkyl, formyl, carboxy, alkoxycarbonyl, C₁-C₈-alkyl-CO-O-, C₁-C₈-alkyl-CO-NH-, or carboxamide. Heteroaryl may be substituted with a mono-oxo to give for example a 4-oxo-1H-quinoline.

The terms "heterocycle," "heterocyclic," and "heterocyclo" refer to an optionally substituted, fully saturated, partially saturated, or non-aromatic cyclic group which is, for example, a 4- to 7-membered monocyclic, 7- to 11-membered bicyclic, or 10- to 15-membered tricyclic ring system, which has at least one heteroatom in at least one carbon atom containing ring. Each ring of the heterocyclic group containing a heteroatom may have 1, 2, or 3 heteroatoms selected from nitrogen atoms, oxygen atoms, and sulfur atoms, where the nitrogen and sulfur heteroatoms may also optionally be oxidized. The nitrogen atoms may optionally be quaternized. The heterocyclic group may be attached at any heteroatom or carbon atom. The heterocyclic group may be substituted by independent replacement of 1 to 3 of the hydrogen atoms thereon with aryl, heteroaryl, halogen, C₁-C₈-alkyl, C₁-C₈-alkoxyl, carboxy, alkoxycarbonyl, or carboxamide.

Exemplary monocyclic heterocyclic groups include pyrrolidinyl; oxetanyl; pyrazolinyl; imidazolinyl; imidazolidinyl; oxazolinyl; oxazolidinyl; isoxazolinyl; thiazolidinyl; isothiazolidinyl; tetrahydrofuryl; piperidinyl; piperazinyl; 2-oxopiperazinyl; 2-oxopiperidinyl; 2-oxopyrrolidinyl; 4-piperidonyl; tetrahydropyranyl; tetrahydrothiopyranyl; tetrahydrothiopyranyl sulfone; morpholinyl; thiomorpholinyl; thiomorpholinyl sulfoxide; thiomorpholinyl sulfone; 1,3-dioxolane; dioxanyl; thietanyl; thiiranyl; 2-oxazepinyl; azepinyl; and the like. Exemplary bicyclic heterocyclic groups include quinuclidinyl; tetrahydroisoquinolinyl; dihydroisoindolyl; dihydroquinazolinyl (such as 3,4-dihydro-4-oxo-quinazolinyl); dihydrobenzofuryl; dihydrobenzothienyl; benzothiopyranyl; dihydrobenzothiopyranyl; dihydrobenzothiopyranyl sulfone;

benzopyranyl; dihydrobenzopyranyl; indolinyl; chromonyl; coumarinyl; isochromanyl; isoindolinyl; piperonyl; tetrahydroquinolinyl; and the like.

Substituted aryl, substituted heteroaryl, and substituted heterocycle may also be substituted with a second substituted aryl, a second substituted heteroaryl, or a second substituted heterocycle to give, for example, a 4-pyrazol-1-yl-phenyl or 4-pyridin-2-yl-phenyl.

The term "carbocyclic" refers to a saturated or unsaturated, non-aromatic, monocyclic, hydrocarbon ring of 3 to 7 carbon atoms.

Designated numbers of carbon atoms (e.g., C₁-C₈ or C₁₋₈) shall refer independently to the number of carbon atoms in an alkyl or cycloalkyl moiety or to the alkyl portion of a larger substituent in which alkyl appears as its prefix root.

The term "hydroxy protecting group" refers to groups known in the art for such purpose. Commonly used hydroxy protecting groups are disclosed, for example, in T. H. Greene and P. G. M. Wuts, *Protective Groups in Organic Synthesis*, 2nd edition, John Wiley & Sons, New York (1991), which is incorporated herein by reference. Illustrative hydroxyl protecting groups include but are not limited to tetrahydropyranyl; benzyl; methylthiomethyl; ethylthiomethyl; pivaloyl; phenylsulfonyl; triphenyl-methyl; trisubstituted silyl such as trimethylsilyl, triethylsilyl, tributylsilyl, tri-isopropylsilyl, t-butyl dimethylsilyl, tri-t-butylsilyl, methyldiphenylsilyl, ethyldiphenylsilyl, t-butyl diphenylsilyl; acyl and aroyl such as acetyl, benzoyl, pivaloylbenzoyl, 4-methoxybenzoyl, 4-nitrobenzoyl and phenylacetyl.

The phrase "a pharmaceutically acceptable salt" denotes one or more salts of the free base or free acid which possess the desired pharmacological activity of the free base or free acid as appropriate and which are neither biologically nor otherwise undesirable. These salts may be derived from inorganic or organic acids. Examples of inorganic acids are hydrochloric acid, nitric acid, hydrobromic acid, sulfuric acid, or phosphoric acid. Examples of organic acids are acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, benzenesulfonic acid, salicylic acid and the like. Suitable salts are furthermore those of inorganic or organic bases, such as KOH,

NaOH, Ca(OH)₂, Al(OH)₃, piperidine, morpholine, ethylamine, triethylamine and the like.

The term "subject" includes, without limitation, any animal or artificially modified animal. As a particular embodiment, the subject is a human.

The term "drug-resistant" or "drug-resistance" refers to the characteristics of a microbe to survive in the presence of a currently available antimicrobial agent such as an antibiotic at its routine, effective concentration.

Unless specified otherwise, it is intended that the definition of any substituent or variable at a particular location in a molecule be independent of its definitions elsewhere in that molecule. It is understood that substituents and substitution patterns on the compounds of this invention can be selected by one of ordinary skill in the art to provide compounds that are chemically stable and that can be readily synthesized by techniques known in the art as well as those methods set forth herein. Further, where a more generic substituent is set forth for any position in the molecules of the present invention, it is understood that the generic substituent may be replaced with more specific substituents, and the resulting molecules are within the scope of the molecules of the present invention.

B. Preparation of Compounds of the Invention

Compounds of the invention may be produced in any manner known in the art. By way of example, compounds of the invention may be prepared according to the following general schemes. The skilled artisan will also recognize the judicious choice of reactions, solvents, and temperatures are an important component in successful synthesis. While the determination of optimal conditions, etc. is routine, it will be understood that a variety of compounds can be generated in a similar fashion, using the guidance of the schemes below.

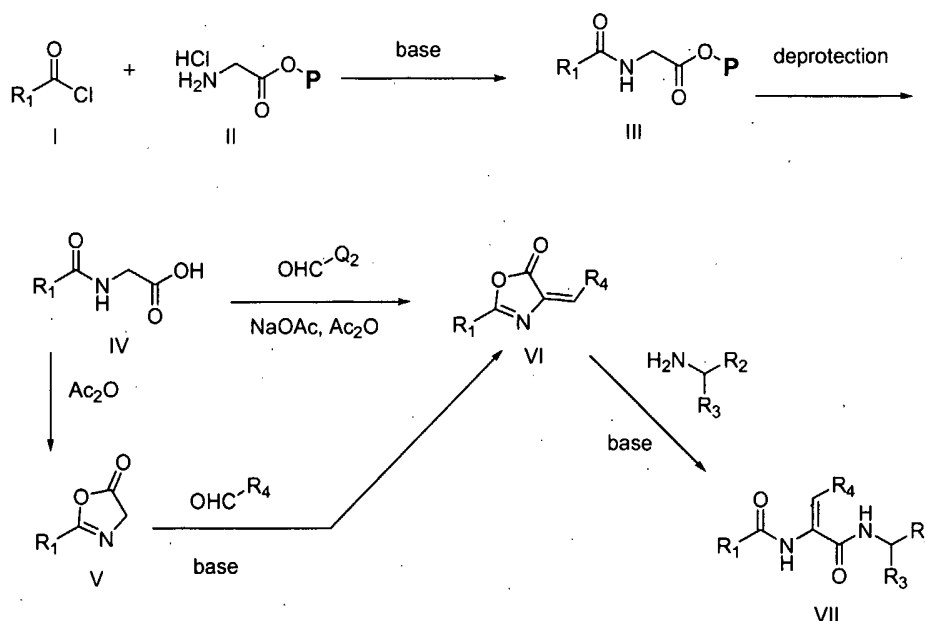
The starting materials used in preparing the compounds of the invention are known, made by published synthetic methods or available from commercial vendors.

It is recognized that the skilled artisan in the art of organic chemistry can readily carry out standard manipulations of the organic compounds without further direction; that is, it is well within the scope and practice of the skilled artisan to carry out such manipulations. These include, but are not limited to, reductions of carbonyl compounds

to their corresponding alcohols, oxidations, acylations, aromatic substitutions, both electrophilic and nucleophilic, etherifications, esterification and saponification and the like. Examples of these manipulations are discussed in standard texts such as March, Advanced Organic Chemistry (Wiley), Carey and Sundberg, Advanced Organic Chemistry (Vol. 2), Feiser & Feiser, Reagents for Organic Synthesis (16 volumes), L. Paquette, Encyclopedia of Reagents for Organic Synthesis (8 volumes), Frost & Fleming, Comprehensive Organic Synthesis (9 volumes) and the like.

The skilled artisan will readily appreciate that certain reactions are best carried out when other functionality is masked or protected in the molecule, thus avoiding any undesirable side reactions and/or increasing the yield of the reaction. Often the skilled artisan utilizes protecting groups to accomplish such increased yields or to avoid the undesired reactions. Examples of these manipulations can be found for example in T. Greene, Protecting Groups in Organic Synthesis.

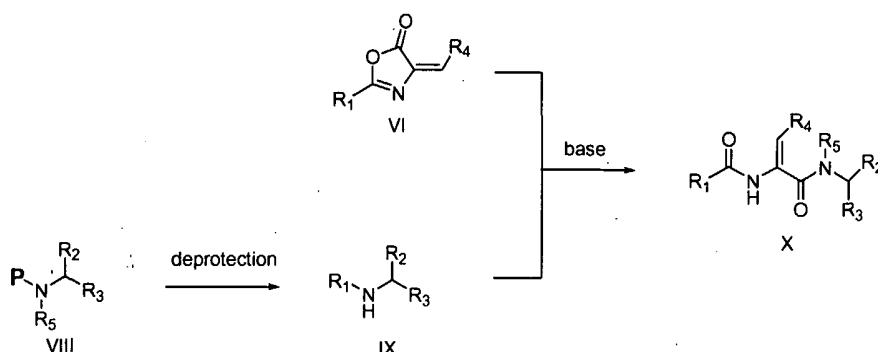
Scheme 1



Dehydropeptides (VII) of formula 1, wherein A is a carbonyl group, Y is NH, R_1 is hydrogen, and Z-E forms a carbon-carbon double bond, can be prepared by the methods outlined in Scheme 1. Reaction of a suitably substituted acid chloride (I) with glycine ester hydrochloride derivative (II), in the presence of a tertiary amine base, such as triethylamine, diisopropyl-ethylamine, or the like, in an inert solvent, such as

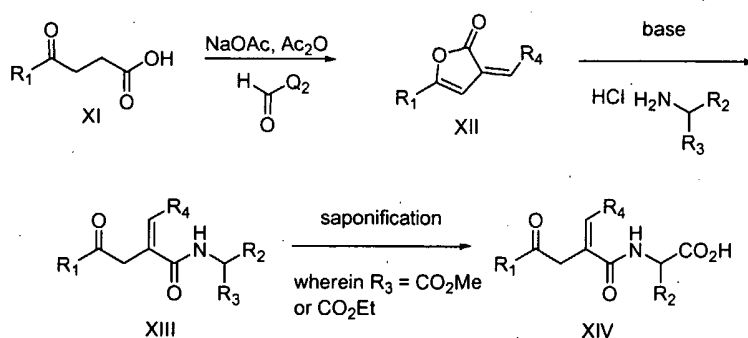
methylene chloride, chloroform, or tetrahydrofuran, for from 1 to 48 hours at a temperature ranging from -20°C to 37°C , affords the corresponding amide derivative (III). Removal of the ester protecting group, for example by treatment with acid, such as formic acid or trifluoroacetic acid, in the case of a t-butyl ester derivative, or by saponification with an alkali metal hydroxide, such as sodium hydroxide or potassium hydroxide, in a suitable solvent, such as tetrahydrofuran, tetrahydrofuran/water mixture, ethanol, methanol, water, or an alcohol/water mixture, at a temperature ranging from 0°C to 80°C for from 1 to 48 hours, in the case of a methyl or ethyl ester derivative, provides the corresponding acid derivative (IV). A host of methods are known in the literature for the formation of the 4H-oxazol-5-one ring system. For example, the 4H-oxazol-5-one derivative (V) may be obtained by heating the acylated amino acetic acid (IV) in acetic anhydride at a temperature ranging from 20°C to 100°C , for from 30 min to 24 hours. Condensation of the 4H-oxazol-5-one (V) with a suitably substituted aldehyde in the presence of a base, such as triethylamine, diisopropyl-ethylamine, and the like, in an inert solvent, such as toluene, benzene, or xylene, at a temperature ranging from 20°C to 110°C for from 1 to 48 hours gives the corresponding benzylidene-4H-oxazol-5-one derivative (VI). Alternatively, VI can be obtained directly from IV by condensation with a suitably substituted aldehyde in the presence of acetic anhydride and a base, such as triethylamine or sodium acetate. This reaction is conducted at a temperature ranging from 20°C to 100°C for from 1 to 48 hours. Finally, dehydropeptide VII can be obtained by reaction of VI with an amine nucleophile, such as an amino acid, in the presence of a base, such as sodium hydroxide or lithium hydroxide, in a suitable solvent, such as aqueous tetrahydrofuran, at a temperature ranging from 20°C to 60°C , for from 1 to 48 hours.

Scheme 2



In the case of the dehydropeptide derivative **X** of formula 1, wherein A is a carbonyl group, Y is NH, R₁ is C₁-C₆ alkyl, and Z - E forms a carbon-carbon double bond or R₁ and R₂ together with the atoms to which they are attached form a pyrrolidine ring, a piperidine ring, or a substituted thiazolidine ring, the route outlined in Scheme 2 can be used in its preparation. A protected amine derivative (**VIII**), as in, for example, a Boc protected N-alkyl amino acid, can be deprotected using standard methods known to those skilled in the art to give the corresponding secondary amine derivative (**IX**). Reaction of the substituted benzylidene-4H-oxazol-5-one intermediate (**VI**), prepared as described in Scheme 1, with the secondary amine derivative (**IX**) in the presence of a base, such as sodium hydroxide or lithium hydroxide, in a suitable solvent, such as aqueous tetrahydrofuran, at a temperature ranging from 20°C to 60°C, for from 1 to 48 hours yields the desired N-alkyl dehydropeptide derivative (**X**).

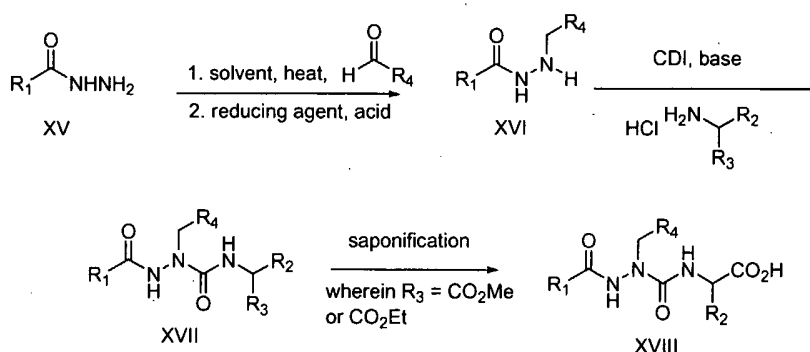
Scheme 3



Analogous to the preparation of the Dehydropeptides (**VII**) illustrated in Scheme 1, compounds (**XIII** and **XIV**) of formula 1, wherein A is a carbonyl group, Y is CH₂, R₁ is hydrogen, and Z-E forms a carbon-carbon double bond, can be prepared by the methods outlined in Scheme 3. Reaction of a suitably substituted 4-oxo-butyric acid

derivative (XI) with a suitably substituted aldehyde in the presence of acetic anhydride and a base, such as sodium acetate, produces the 3H-furan-2-one derivative (XII). Reaction of XII with a suitably substituted amine nucleophile, such as an amino acid ester hydrochloride derivative, in the presence of a base, such as triethylamine, diisopropyl-ethylamine or the like, affords the corresponding α,β -unsaturated amide derivative XIII. In the case where R₂ is an ester functionality, such as CO₂Me or CO₂Et, saponification with an alkali metal hydroxide, such as sodium hydroxide, lithium hydroxide or potassium hydroxide, in a suitable solvent, such as tetrahydrofuran, tetrahydrofuran/water mixture, ethanol, methanol, water, or an alcohol/water mixture, at a temperature ranging from 0°C to 80°C for from 1 to 48 hours, provides the corresponding acid derivative (XIV).

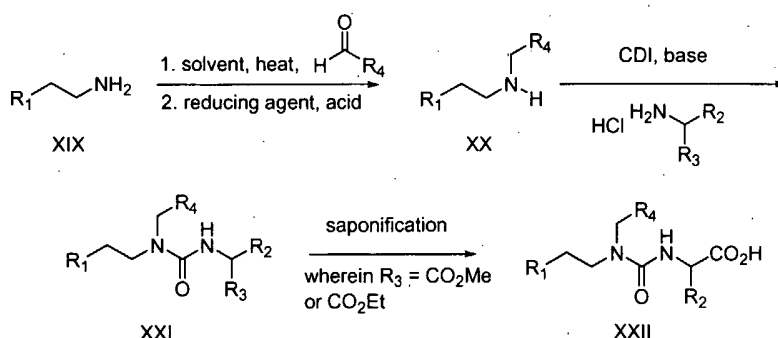
Scheme 4



Compounds (XVII and XVIII) of formula 1, wherein A is a carbonyl group, Y is NH, R₁ is hydrogen, and Z-E forms a carbon-nitrogen single bond, can be prepared by the methods outlined in Scheme 4. Reaction of a suitably substituted hydrazide derivative (XV) with a suitably substituted aldehyde in an appropriate solvent such as methanol or ethanol at a temperature ranging from 20°C to 80°C for from 1 to 48 hours provides the corresponding hydrazone derivative, which may be converted in a subsequent step to the corresponding N-alkylhydrazide derivative (XVI) by treatment with a suitable reducing agent, such as triethylsilane, sodium borohydride or sodium cyanoborohydride, in the presence of an acid, such as trifluoroacetic acid, acetic acid or hydrochloric acid, in a suitable solvent such as dichloromethane, methanol or ethanol for from 1 to 48 hours as is appropriate to effect reduction. Alternatively, the conversion of XV to XVI may be conducted in a single pot by combining hydrazide XV with an aldehyde in the presence

of a suitable reducing agent and acid. Conversion of hydrazide **XVI** to urea derivative **XVII** can be carried out by reaction with an amine nucleophile, such as an amino acid ester hydrochloride, and an acylating agent, such as carbonyl diimidazole or triphosgene, in the presence of a suitable base, such as triethylamine, diisopropyl-ethylamine, or the like, in an inert solvent, such as dichloromethane, chloroform, or tetrahydrofuran. The reaction is conducted at a temperature from -20°C to 37°C for from 2 to 48 hours. In the case where R_2 is an ester functionality, such as CO_2Me or CO_2Et , saponification with an alkali metal hydroxide, such as sodium hydroxide, lithium hydroxide or potassium hydroxide, in a suitable solvent, such as tetrahydrofuran, tetrahydrofuran/water mixture ethanol, methanol, water, or an alcohol/water mixture, at a temperature ranging from 0°C to 80°C for from 1 to 48 hours, provides the corresponding acid derivative (**XVIII**).

Scheme 5



Compounds (**XXI** and **XXII**) of formula 1, wherein A is CH_2 , Y is CH_2 , R_1 is hydrogen, and Z-E forms a carbon-nitrogen single bond, can be prepared by the methods outlined in Scheme 5. Reaction of a suitably substituted amine derivative (**XIX**) with a suitably substituted aldehyde in an appropriate solvent such as methanol or ethanol at a temperature ranging from 20°C to 80°C for from 1 to 48 hours provides the corresponding imine derivative, which may be converted in a subsequent step to the corresponding secondary amine derivative (**XX**) by treatment with a suitable reducing agent, such as triethylsilane, sodium borohydride or sodium cyanoborohydride, in the presence of an acid, such as trifluoroacetic acid, acetic acid or hydrochloric acid, in a suitable solvent such as dichloromethane, methanol or ethanol for from 1 to 48 hours as is appropriate to effect reduction. Alternatively, the conversion of **XIX** to **XX** may be conducted in a single pot by combining amine **XIX** with an aldehyde in the presence of a

suitable reducing agent and acid. Conversion of amine **XX** to urea derivative **XXI** can be carried out by reaction with an amine nucleophile, such as an amino acid ester hydrochloride, and an acylating agent, such as carbonyldiimidazole or triphosgene, in the presence of a suitable base, such as triethylamine, diisopropyl-ethylamine, or the like, in an inert solvent, such as dichloromethane, chloroform, or tetrahydrofuran. The reaction is conducted at a temperature from -20°C to 37°C for from 2 to 48 hours. In the case where R_2 is an ester functionality, such as CO_2Me or CO_2Et , saponification with an alkali metal hydroxide, such as sodium hydroxide, lithium hydroxide or potassium hydroxide, in a suitable solvent, such as tetrahydrofuran, tetrahydrofuran/water mixture, ethanol, methanol, water, or an alcohol/water mixture, at a temperature ranging from 0°C to 80°C for from 1 to 48 hours, provides the corresponding acid derivative (**XXII**).

In certain preferred embodiments, compounds of the invention may be resolved to enantiomerically pure compositions or synthesized as enantiomerically pure compositions using any method known in art. By way of example, compounds of the invention may be resolved by direct crystallization of enantiomer mixtures, by diastereomer salt formation of enantiomers, by the formation and separation of diastereomers or by enzymatic resolution of a racemic mixture.

These and other reaction methodologies may be useful in preparing the compounds of the invention, as recognized by one of skill in the art. Various modifications to the above schemes and procedures will be apparent to one of skill in the art, and the invention is not limited specifically by the method of preparing the compounds of the invention.

C. Methods of the Invention

In another aspect of the invention, methods are provided for the inhibition of Type III protein secretion, and/or the treatment and prevention of bacterial infection, particularly Gram-negative bacterial infection using the compounds described herein.

In one embodiment, the invention is directed to methods for inhibiting Type III protein secretion comprising administering a secretion-inhibiting amount of at least one compound of the invention to a subject in need thereof.

In yet another embodiment, methods for treating or prevention of bacterial infection, particularly Gram-Negative bacterial infection are provided comprising administering a therapeutically or prophylactically effective amount of at least one compound of the invention to a subject in need thereof.

According to the methods of the invention, the compound(s) may be administered to the subject via any drug delivery route known in the art. Specific exemplary administration routes include oral, ocular, rectal, buccal, topical, nasal, ophthalmic, subcutaneous, intramuscular, intravenous (bolus and infusion), intracerebral, transdermal, and pulmonary.

The terms "secretion-inhibiting amount", "therapeutically effective amount", and "prophylactically effective amount", as used herein, refer to an amount of a compound of the invention sufficient to treat, ameliorate, or prevent the identified disease or condition, or to exhibit a detectable therapeutic, prophylactic, or inhibitory effect. The effect can be detected by, for example, the assays disclosed in the following examples. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition; and the therapeutic or combination of therapeutics selected for administration. Therapeutically and prophylactically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician.

For any compound, the therapeutically or prophylactically effective amount can be estimated initially either in cell culture assays, *e.g.*, of neoplastic cells, or in animal models, usually rats, mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. Therapeutic/prophylactic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, ED₅₀/LD₅₀. Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies may be used in formulating a range of dosage for human use. The

dosage contained in such compositions is preferably within a range of circulating concentrations that include an ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

More specifically, the concentration-biological effect relationships observed with regard to the compound(s) of the present invention indicate an initial target plasma concentration ranging from approximately 5 µg/mL to approximately 100 µg/mL, preferably from approximately 10 µg/mL to approximately 100 µg/mL, more preferably from approximately 20 µg/mL to approximately 100 µg/mL. To achieve such plasma concentrations, the compounds of the invention may be administered at doses that vary from 0.1 µg to 100,000 mg, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and is generally available to practitioners in the art. In general the dose will be in the range of about 1 mg/day to about 10g/day, or about 0.1g to about 3g/day, or about 0.3g to about 3g/day, or about 0.5g to about 2g/day, in single, divided, or continuous doses for a patient weighing between about 40 to about 100 kg (which dose may be adjusted for patients above or below this weight range, particularly children under 40 kg).

The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active agent(s) or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

D. Metabolites of the Compounds of the Invention

Also falling within the scope of the present invention are the *in vivo* metabolic products of the compounds described herein. Such products may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, the

invention includes compounds produced by a process comprising contacting a compound of this invention with a mammalian tissue or a mammal for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radio-labeled (*e.g.* ^{14}C or ^3H) compound of the invention, administering it in a detectable dose (*e.g.*, greater than about 0.5 mg/kg) to a mammal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours), and isolating its conversion products from urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, *e.g.*, by MS or NMR analysis. In general, analysis of metabolites may be done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found *in vivo*, are useful in diagnostic assays for therapeutic dosing of the compounds of the invention even if they possess no biological activity of their own.

E. Pharmaceutical Compositions of the Invention

While it is possible for the compounds of the present invention to be administered neat, it may be preferable to formulate the compounds as pharmaceutical compositions. As such, in yet another aspect of the invention, pharmaceutical compositions useful in the methods of the invention are provided. The pharmaceutical compositions of the invention may be formulated with pharmaceutically acceptable excipients such as carriers, solvents, stabilizers, adjuvants, diluents, *etc.*, depending upon the particular mode of administration and dosage form. The pharmaceutical compositions should generally be formulated to achieve a physiologically compatible pH, and may range from a pH of about 3 to a pH of about 11, preferably about pH 3 to about pH 7, depending on the formulation and route of administration. In alternative embodiments, it may be preferred that the pH is adjusted to a range from about pH 5.0 to about pH 8.0.

More particularly, the pharmaceutical compositions of the invention comprise a therapeutically or prophylactically effective amount of at least one compound of the present invention, together with one or more pharmaceutically acceptable excipients. Optionally, the pharmaceutical compositions of the invention may comprise a

combination of compounds of the present invention, or may include a second active ingredient useful in the treatment or prevention of bacterial infection (e.g., anti-bacterial or anti-microbial agents).

Formulations of the present invention, *e.g.*, for parenteral or oral administration, are most typically solids, liquid solutions, emulsions or suspensions, while inhalable formulations for pulmonary administration are generally liquids or powders, with powder formulations being generally preferred. A preferred pharmaceutical composition of the invention may also be formulated as a lyophilized solid that is reconstituted with a physiologically compatible solvent prior to administration. Alternative pharmaceutical compositions of the invention may be formulated as syrups, creams, ointments, tablets, and the like.

The term "pharmaceutically acceptable excipient" refers to an excipient for administration of a pharmaceutical agent, such as the compounds of the present invention. The term refers to any pharmaceutical excipient that may be administered without undue toxicity. Pharmaceutically acceptable excipients are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there exists a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g.*, Remington's Pharmaceutical Sciences).

Suitable excipients may be carrier molecules that include large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Other exemplary excipients include antioxidants such as ascorbic acid; chelating agents such as EDTA; carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkyl-methylcellulose, stearic acid; liquids such as oils, water, saline, glycerol and ethanol; wetting or emulsifying agents; pH buffering substances; and the like. Liposomes are also included within the definition of pharmaceutically acceptable excipients.

The pharmaceutical compositions of the invention may be formulated in any form suitable for the intended method of administration. When intended for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, non-aqueous solutions,

dispersible powders or granules (including micronized particles or nanoparticles), emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation.

Pharmaceutically acceptable excipients particularly suitable for use in conjunction with tablets include, for example, inert diluents, such as celluloses, calcium or sodium carbonate, lactose, calcium or sodium phosphate; disintegrating agents, such as croscarmellose sodium, cross-linked povidone, maize starch, or alginic acid; binding agents, such as povidone, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example celluloses, lactose, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with non-aqueous or oil medium, such as glycerin, propylene glycol, polyethylene glycol, peanut oil, liquid paraffin or olive oil.

In another embodiment, pharmaceutical compositions of the invention may be formulated as suspensions comprising a compound of the present invention in admixture with at least one pharmaceutically acceptable excipient suitable for the manufacture of a suspension. In yet another embodiment, pharmaceutical compositions of the invention may be formulated as dispersible powders and granules suitable for preparation of a suspension by the addition of suitable excipients.

Excipients suitable for use in connection with suspensions include suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, dispersing or wetting agents such as a naturally occurring phosphatide (*e.g.*, lecithin), a

condensation product of an alkylene oxide with a fatty acid (*e.g.*, polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (*e.g.*, heptadecaethyleneoxycethanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene sorbitan monooleate); and thickening agents, such as carbomer, beeswax, hard paraffin or cetyl alcohol. The suspensions may also contain one or more preservatives such as acetic acid, methyl and/or *n*-propyl *p*-hydroxy-benzoate; one or more coloring agents; one or more flavoring agents; and one or more sweetening agents such as sucrose or saccharin.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth; naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids; hexitol anhydrides, such as sorbitan monooleate; and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

Additionally, the pharmaceutical compositions of the invention may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous emulsion or oleaginous suspension. This emulsion or suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,2-propane-diol. The sterile injectable preparation may also be prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile fixed oils may be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides.

In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

Generally, the compounds of the present invention useful in the methods of the present invention are substantially insoluble in water and are sparingly soluble in most pharmaceutically acceptable protic solvents and in vegetable oils. However, the compounds are generally soluble in medium chain fatty acids (*e.g.*, caprylic and capric acids) or triglycerides and have high solubility in propylene glycol esters of medium chain fatty acids. Also contemplated in the invention are compounds which have been modified by substitutions or additions of chemical or biochemical moieties which make them more suitable for delivery (*e.g.*, increase solubility, bioactivity, palatability, decrease adverse reactions, *etc.*), for example by esterification, glycosylation, PEGylation, *etc.*

In a preferred embodiment, the compounds of the present invention may be formulated for oral administration in a lipid-based formulation suitable for low solubility compounds. Lipid-based formulations can generally enhance the oral bioavailability of such compounds. As such, a preferred pharmaceutical composition of the invention comprises a therapeutically or prophylactically effective amount of a compound of the present invention, together with at least one pharmaceutically acceptable excipient selected from the group consisting of: medium chain fatty acids or propylene glycol esters thereof (*e.g.*, propylene glycol esters of edible fatty acids such as caprylic and capric fatty acids) and pharmaceutically acceptable surfactants such as polyoxyl 40 hydrogenated castor oil.

In an alternative preferred embodiment, cyclodextrins may be added as aqueous solubility enhancers. Preferred cyclodextrins include hydroxypropyl, hydroxyethyl, glucosyl, maltosyl and maltotriosyl derivatives of α -, β -, and γ -cyclodextrin. A particularly preferred cyclodextrin solubility enhancer is hydroxypropyl- β -cyclodextrin (HPBC), which may be added to any of the above-described compositions to further improve the aqueous solubility characteristics of the compounds of the present invention. In one embodiment, the composition comprises 0.1% to 20% hydroxypropyl- β -cyclodextrin, more preferably 1% to 15% hydroxypropyl- β -cyclodextrin, and even more preferably from 2.5% to 10% hydroxypropyl- β -cyclodextrin. The amount of solubility

enhancer employed will depend on the amount of the compound of the present invention in the composition.

F. Combination Therapy

It is also possible to combine any compound of the present invention with one or more other active ingredients useful in the treatment or prevention of bacterial infection, including compounds, in a unitary dosage form, or in separate dosage forms intended for simultaneous or sequential administration to a patient in need of treatment. When administered sequentially, the combination may be administered in two or more administrations. In an alternative embodiment, it is possible to administer one or more compounds of the present invention and one or more additional active ingredients by different routes.

The skilled artisan will recognize that a variety of active ingredients may be administered in combination with the compounds of the present invention that may act to augment or synergistically enhance the Type III protein secretion-inhibiting activity of the compounds of the invention.

According to the methods of the invention, the combination of active ingredients may be: (1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by any other combination therapy regimen known in the art. When delivered in alternation therapy, the methods of the invention may comprise administering or delivering the active ingredients sequentially, *e.g.*, in separate solution, emulsion, suspension, tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, *i.e.*, serially, whereas in simultaneous therapy, effective dosages of two or more active ingredients are administered together. Various sequences of intermittent combination therapy may also be used.

To assist in understanding the present invention, the following Examples are included. The experiments relating to this invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are

considered to fall within the scope of the invention as described herein and hereinafter claimed.

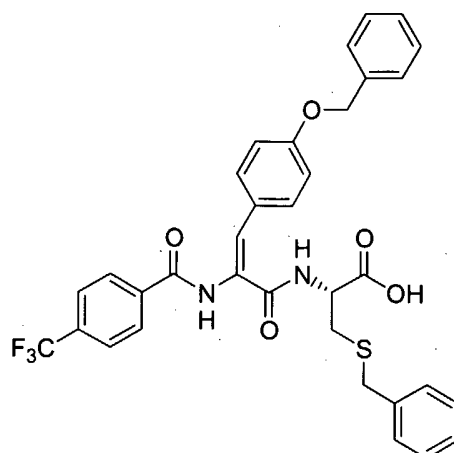
EXAMPLES

The present invention is described in more detail with reference to the following non-limiting examples, which are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof. The examples illustrate the preparation of certain compounds of the invention, and the testing of these compounds *in vitro* and/or *in vivo*. Those of skill in the art will understand that the techniques described in these examples represent techniques described by the inventors to function well in the practice of the invention, and as such constitute preferred modes for the practice thereof. However, it should be appreciated that those of skill in the art should in light of the present disclosure, appreciate that many changes can be made in the specific methods that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

The following examples describe in detail the chemical synthesis of representative compounds of the present invention. The procedures are illustrations, and the invention should not be construed as being limited by the chemical reactions and conditions they express. No attempt has been made to optimize the yields obtained in these reactions, and it would be obvious to one skilled in the art that variations in reaction times, temperatures, solvents, and/or reagents could increase the yields.

Example 1: Preparation of Compounds of the Invention

Compounds of Formula I may be prepared according to the schemes disclosed herein as follows.

Compound 1

(R)-2-[3-(4-Benzyloxyphenyl)-2-(4-trifluoromethylbenzoylamino)acryloylamino]-3-benzylsulfanylpropionic acid

Step A: (4-Trifluoromethylbenzoylamino)acetic acid tert-butyl ester

To a solution of 4-trifluoromethylbenzoyl chloride (15.0 mL, 0.1 mol) and glycine tert-butyl ester hydrochloride (17.8 g, 0.1 mol) in dichloromethane (200.0 mL) at 0 °C, was added triethylamine (29.6 mL, 0.2 mol) and the reaction mixture was stirred at 0 °C for 30 min and warmed to room temperature for 3-20 h. The mixture was washed with water, 1N hydrochloric acid and water. The organic layer was separated, dried over sodium sulfate, filtered and the filtrate was concentrated in vacuo to give 28.7 g of the desired product as a white solid (100%), which was used without further purification.

Step B: (4-Trifluoromethylbenzoylamino)acetic acid

(4-Trifluoromethylbenzoylamino)acetic acid tert-butyl ester from Step A (28.0 g, 0.1 mol) was treated with formic acid (80.0 mL) at room temperature for 14 h. Excess formic acid was removed under reduced pressure affording 22 g of the title compound as an off-white solid (100%), which was used without further purification.

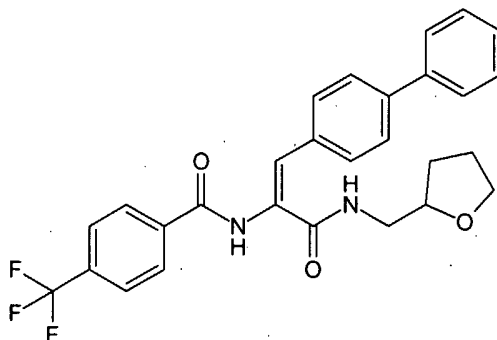
Step C: 4-(4-Benzyloxy benzylidene)-2-(4-trifluoromethylphenyl)-4H-oxazol-5-one

The mixture of (4-trifluoromethylbenzoylamino)acetic acid from Step B (494 mg, 2.0 mmol), 4-benzyloxy benzaldehyde (425 mg, 2.0 mmol) and sodium acetate (492 mg, 6.0 mmol) in acetic anhydride (2.0 mL) was heated at 70 °C for 2 h. The reaction mixture was cooled to room temperature and the product was isolated by filtration (470 mg, 56%).

Step D: 2-[3-(4-Benzoyloxyphenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]-3-benzylsulfanylpropionic acid

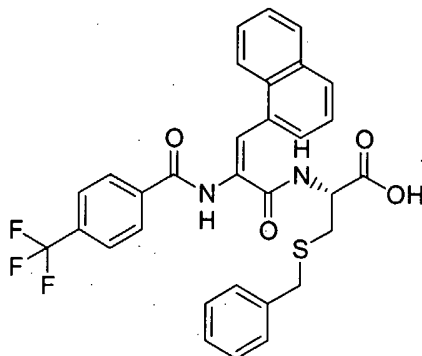
To a solution of 4-(4-benzyloxy benzylidene)-2-(4-trifluoromethyl-phenyl)-4H-oxazol-5-one from Step C (100 mg, 0.24 mmol) in THF (1.0 mL) was added a solution of H-Cys(Bzl)-L-OH (50 mg, 0.24 mmol) in a mixture of lithium hydroxide (aq., 1N, 0.24 mL) and water (1.0 mL). The reaction mixture was stirred at room temperature for 6 h and concentrated in vacuo. The remaining aqueous layer was acidified with 1N HCl and the light yellow precipitate was collected by filtration giving 68 mg of the title compound (45%). MS 635.1 (M+H)⁺.

Compound 2



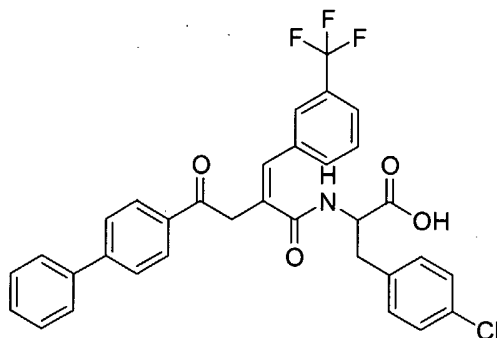
N-{2-Biphenyl-4-yl-1-[(tetrahydro-furan-2-yl-methyl)carbonyl]vinyl}-4-trifluoromethylbenzamide

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-biphenylcarboxaldehyde for the 4-benzyloxy-benzaldehyde of Step B of Compound 1; and by substituting tetrahydro-furan-2-yl-methylamine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 495.2 (M+H)⁺.

Compound 3

(R)-3-Benzylsulfanyl-2-[3-naphthalen-1-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting naphthalene-1-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 577.2 (M-H)⁻.

Compound 4

2-[2-(2-Biphenyl-4-yl-2-oxoethyl)-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

Step A: 5-Biphenyl-4-yl-3-(3-trifluoromethyl benzylidene)-3H-furan-2-one

A suspension of 4-biphenyl-4-yl-4-oxobutyric acid (508.6 mg, 2.0 mmol), 3-trifluoromethyl benzaldehyde (0.27 mL, 2.0 mmol) and sodium acetate (164.0 mg, 2.0 mmol) in acetic anhydride (1.2 mL) was heated at 90°C for 2 h. The reaction mixture was cooled to room temperature and diluted with water. The precipitate was collected by filtration giving 450 mg of the desired product (57%), which was used without further purification.

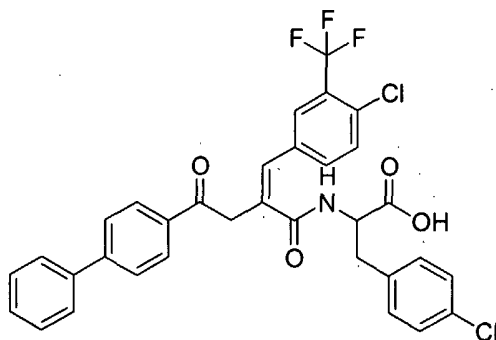
Step B: 2-[2-(2-Biphenyl-4-yl-2-oxoethyl)-3-(3-trifluoromethylphenyl)acryloyl-amino]-3-(4-chlorophenyl)propionic acid ethyl ester

A mixture of 5-biphenyl-4-yl-3-(3-trifluoromethyl benzylidene)-3H-furan-2-one from Step A (75.6 mg, 0.2 mmol), D,L-4-chlorophenylalanine ethyl ester hydrochloride (50.7 mg, 0.2 mmol) and triethylamine (0.027 mL, 0.2 mmol) in toluene (2.0 mL) was heated at 90°C for 14 h. The reaction mixture was cooled to room temperature and diluted with ethyl acetate (3.0 mL). After washing with 1N HCl and water, the organic layer was separated and concentrated in vacuo. Purification by medium pressure liquid chromatography on silica gel (1:9 ethyl acetate/hexanes) gave 51 mg (43%) of the title compound.

Step C: 2-[2-(2-Biphenyl-4-yl-2-oxoethyl)-3-(3-trifluoromethylphenyl)acryloyl-amino]-3-(4-chlorophenyl)propionic acid

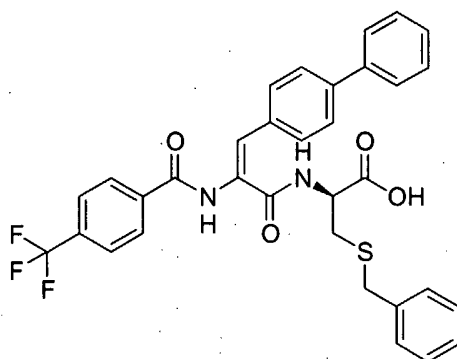
To a solution of 2-[2-(2-biphenyl-4-yl-2-oxoethyl)-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid ethyl ester from Step B (45 mg, 0.07 mmol) in THF (1.0 mL) was added 1N sodium hydroxide (aq., 1.0 mL) and the reaction mixture was stirred at room temperature for 3 h. After acidification with 1N HCl to pH 2, the solid was collected by filtration giving 35 mg (84%) of the title compound. MS 596.0 (M+H)⁺.

Compound 5



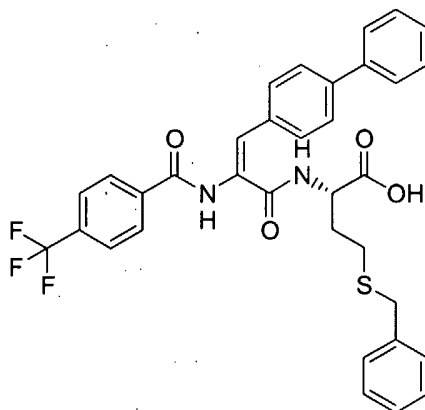
2-[2-(2-Biphenyl-4-yl-2-oxoethyl)-3-(4-chloro-3-trifluoromethylphenyl)-acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 4 by substituting 4-chloro-3-trifluoromethyl benzaldehyde for the 3-trifluoromethyl benzaldehyde of Step A of Compound 4. MS 624.1 (M-H)⁻.

Compound 6

(S)-3-Benzylsulfanyl-2-[3-biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

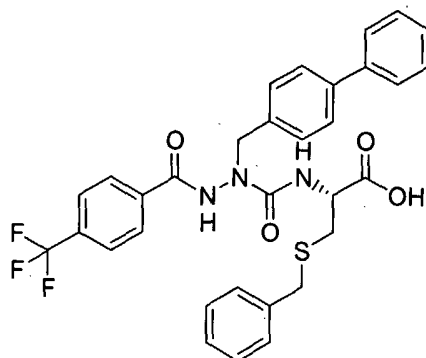
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting H-Cys(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 603.2 (M-H)⁻.

Compound 7

(S)-4-Benzylsulfanyl-2-[3-biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]butyric acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting homo-H-Cys(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 619.2 (M+H)⁺.

Compound 8



4-Trifluoromethylbenzoic acid-N'-[(biphenyl-4-yl)methyl]-N'-[[[(2-benzylthio)-1-carboxyethyl]amino]carbonyl]hydrazide

Step A: 4-Trifluoromethylbenzoyl acid biphenyl-4-yl-methylene hydrazide

A mixture of 4-trifluoromethylbenzoic acid hydrazide (1.0 g, 5.0 mmol) and biphenyl-4-carboxaldehyde (0.9 g, 5.0 mmol) in methanol (20.0 mL) was heated at reflux temperature for 3 h. The reaction mixture was cooled to room temperature and concentrated in vacuo giving the title compound in quantitative yield, which was used without further purification.

Step B: 4-Trifluoromethylbenzoic acid N'-biphenyl-4-yl-methyl hydrazide hydrochloride

To a suspension of 4-trifluoromethylbenzoic acid biphenyl-4-yl-methylene hydrazide from Step A (740 mg, 2.0 mmol) in trifluoroacetic acid (3.0 mL) was added triethylsilane (0.64 mL, 4.0 mmol) slowly at room temperature and the reaction mixture was stirred for 15 h. The mixture was acidified with 1N HCl and the precipitate was collected by filtration giving 740 mg (100%) of the title compound.

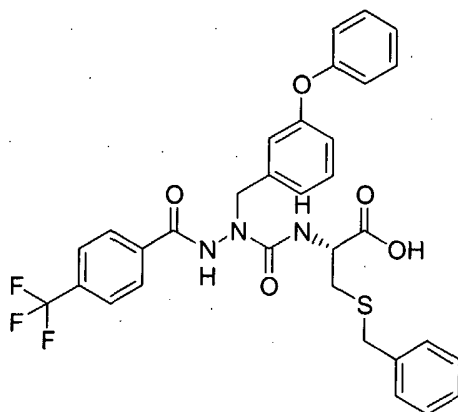
Step C: 4-Trifluoromethylbenzoic acid-N'-[(biphenyl-4-yl)methyl]-N'-[[[(2-benzylthio)-(1-methoxycarbonyl)ethyl]amino]carbonyl]hydrazide

To a solution of S-benzyl-L-cysteine methyl ester hydrochloride (100 mg, 0.38 mmol), carbonyldiimidazole (68 mg, 0.42 mmol) and triethylamine (0.32 mL, 2.28 mmol) in dichloromethane (8.0 mL) was added 4-trifluoromethylbenzoic acid N'-biphenyl-4-yl-methyl hydrazide hydrochloride from Step B (155 mg, 0.38 mmol), and the reaction mixture was stirred at room temperature for 14 h. It was then washed with water and the organic layer was separated, dried over sodium sulfate and concentrated in vacuo. Purification by medium pressure liquid chromatography on silica gel (1:9 ethyl acetate/hexanes) gave 120 mg (50%) of the title compound.

Step D: 4-Trifluoromethylbenzoic acid-N'-[(biphenyl-4-yl)methyl]-N'-[[[(2-benzylthio)-1-carboxyethyl]amino]carbonyl]hydrazide

The product from Step C (97 mg, 0.16 mmol) was dissolved in THF (1.0 mL) and treated with 1N sodium hydroxide (1.0 mL) at room temperature for 3 h. The reaction mixture was acidified with 1N HCl to pH 2-3. After removal of THF under reduced pressure, the product was diluted with dichloromethane and washed with water multiple times. The organic layer was separated, dried over sodium sulfate and concentrated in vacuo to give 77 mg (81%) of the title compound. MS 608.2 (M+H)⁺.

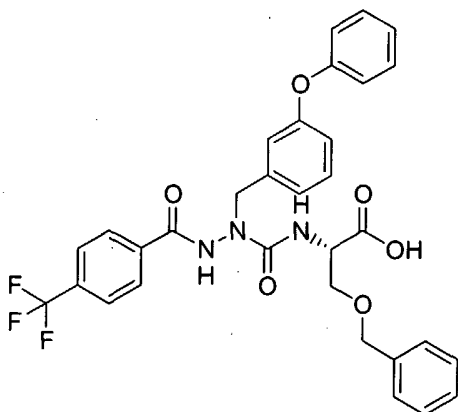
Compound 9



4-Trifluoromethylbenzoic acid-N'-[(3-phenoxyphenyl)methyl]-N'-[[[(2-benzylthio)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting 3-phenoxybenzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8. MS 624.1 (M+H)⁺.

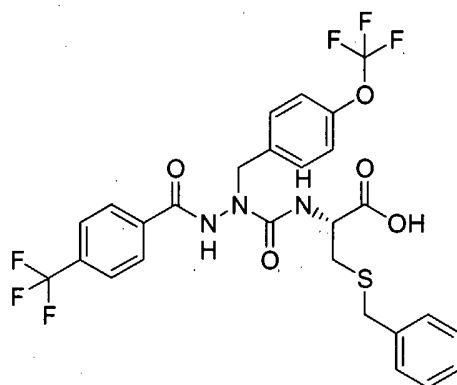
Compound 10



4-Trifluoromethylbenzoic acid-N'-[(3-phenoxyphenyl)methyl]-N'-[[[(2-benzyloxy)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting 3-phenoxybenzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8; and by substituting O-benzyl-L-serine methyl ester hydrochloride for the S-benzyl-L-cysteine methyl ester hydrochloride of Step C of Compound 8. MS 608.1 (M+H)⁺.

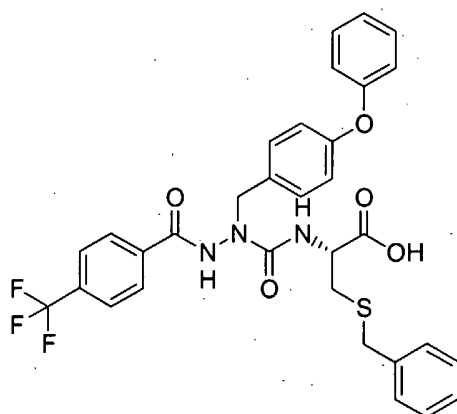
Compound 11



4-Trifluoromethylbenzoic acid-N'-[(4-trifluoromethoxyphenyl)methyl]-N'-[[[(2-benzylthio)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting 4-trifluoromethoxy benzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8. MS 616.0 (M+H)⁺.

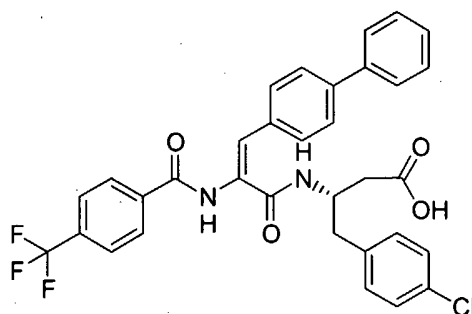
Compound 12



4-Trifluoromethylbenzoic acid-N'-[(4-phenoxyphenyl)methyl]-N'-[[[(2-benzylthio)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting 4-phenoxybenzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8. MS 624.1 (M+H)⁺.

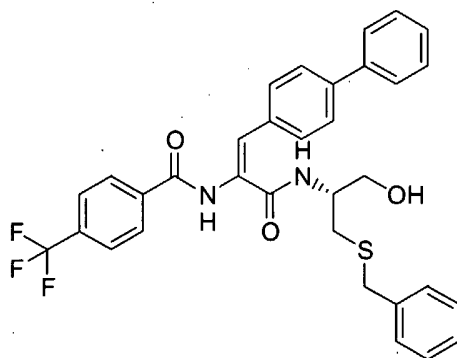
Compound 13



(S)-3-[3-Biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)acryloylamino]-4-(4-chlorophenyl)butyric acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting L-3-amino-4-(4-chloro-phenyl)butyric acid for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 605.1 (M-H)⁻.

Compound 14

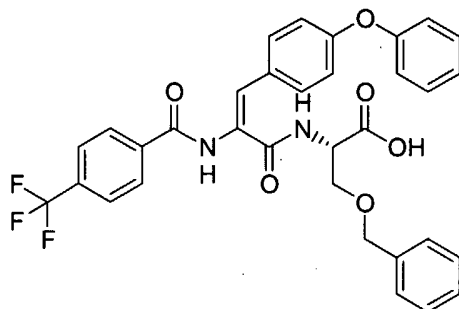


(R)-N-[1-(1-Benzylsulfanyl-methyl)-2-hydroxyethylcarbonyl]-2-biphenyl-4-yl-vinyl]-4-trifluoromethylbenzamide

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C

of Compound 1; and by substituting 2-amino-3-benzylsulfanylpropan-1-ol for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 591.2 (M-H)⁻.

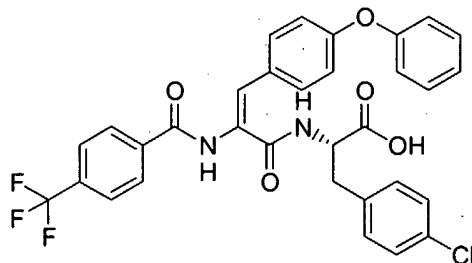
Compound 15



(S)-3-Benzoyloxy-2-[3-(4-phenoxyphenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

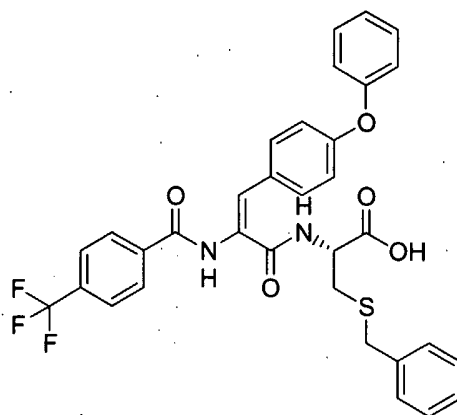
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 603.2 (M-H)⁻.

Compound 16



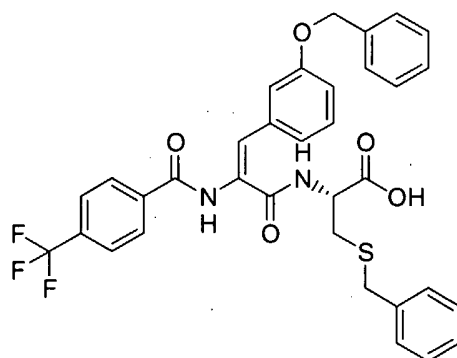
(S)-3-(4-Chlorophenyl)-2-[3-(4-phenoxyphenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 609.2 (M+H)⁺.

Compound 17

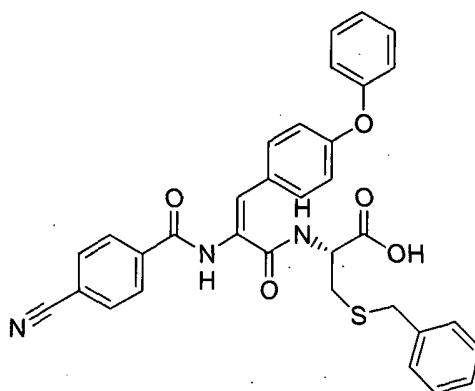
(R)-3-Benzylsulfanyl-2-[3-(4-phenoxyphenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 621.2 (M+H)⁺.

Compound 18

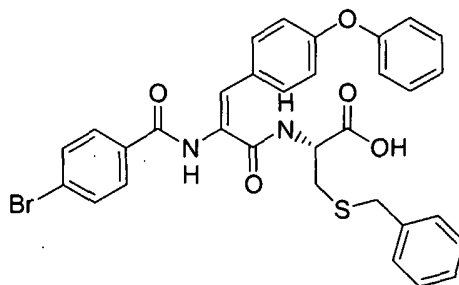
(R)-2-[3-(3-Benzzyloxyphenyl)-2-(4-trifluoromethylbenzoylamino)acryloylamino]-3-benzylsulfanylpropionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 3-benzyloxy benzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 635.1 (M+H)⁺.

Compound 19

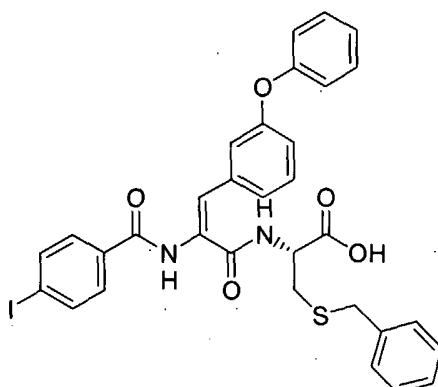
(R)-3-Benzylsulfanyl-2-[2-(4-cyanobenzoylamino)-3-(4-phenoxyphenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-cyanobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-phenoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 578.1 (M+H)⁺.

Compound 20

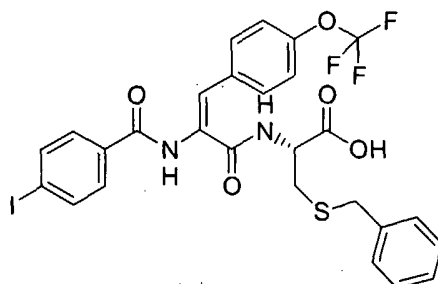
(R)-3-Benzylsulfanyl-2-[2-(4-bromobenzoylamino)-3-(4-phenoxyphenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-bromobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-phenoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 633.0 (M+H)⁺.

Compound 21

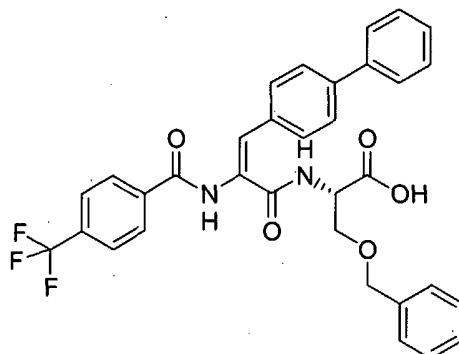
(R)-3-Benzylsulfanyl-2-[2-(4-iodobenzoylamino)-3-(3-phenoxyphenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-iodobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-phenoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 679.0 (M+H)⁺.

Compound 22

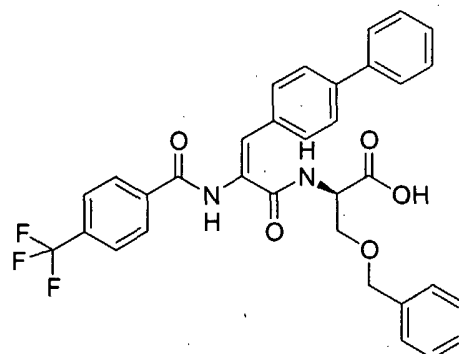
(R)-3-Benzylsulfanyl-2-[2-(4-iodobenzoylamino)-3-(4-trifluoromethoxyphenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-iodobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 670.9 (M+H)⁺.

Compound 23

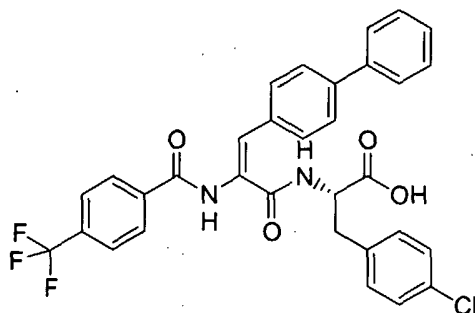
(S)-3-Benzyloxy-2-[3-biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 589.2 (M+H)⁺.

Compound 24

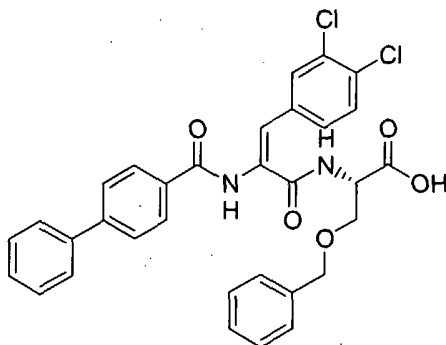
(R)-3-Benzyloxy-2-[3-biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 589.2 (M+H)⁺.

Compound 25

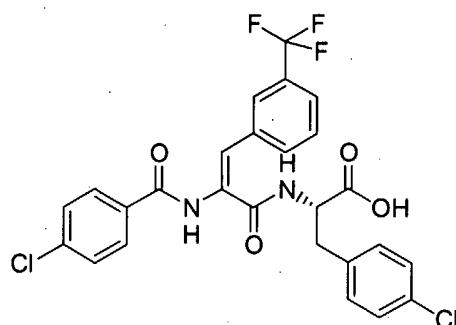
(S)-2-[3-Biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 593.2 (M+H)⁺.

Compound 26

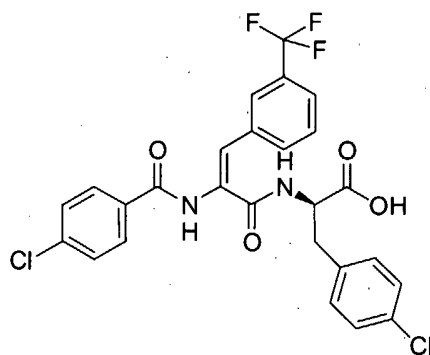
(S)-3-Benzyloxy-2-[2-[(biphenyl-4-carbonyl)amino]-3-(3,4-dichlorophenyl)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichloro-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 587.2 (M-H)⁻.

Compound 27

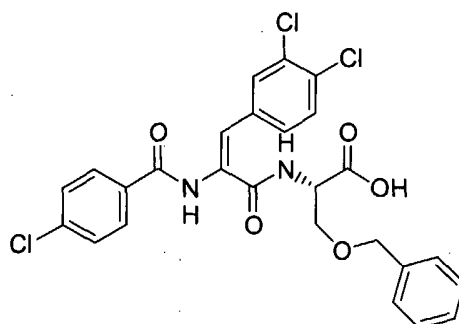
(S)-2-[2-(4-Chlorobenzoylamino)-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-chlorobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 549.1 (M-H).

Compound 28

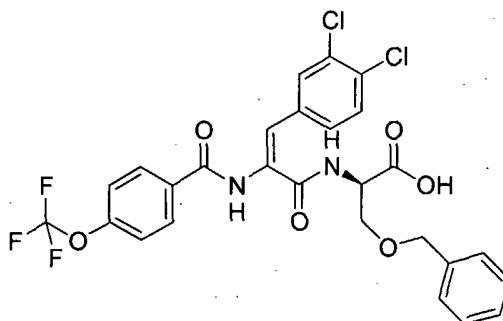
(R)-2-[2-(4-Chlorobenzoylamino)-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-chlorobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 549.1 (M-H).

Compound 29

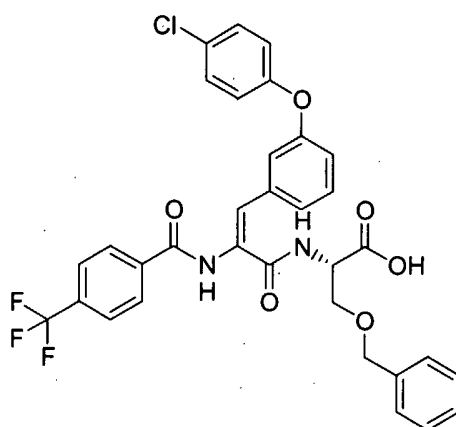
(S)-3-Benzyloxy-2-[2-(4-chlorobenzoylamino)-3-(3,4-dichlorophenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-chlorobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichloro-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 547.0 (M-H).

Compound 30

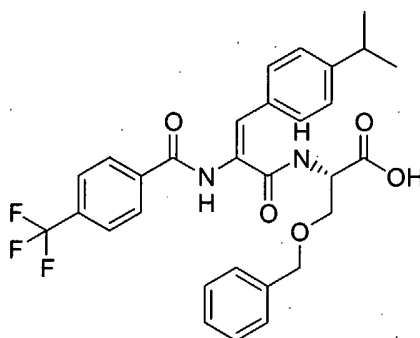
(R)-3-Benzyloxy-2-[3-(3,4-dichlorophenyl)-2-(4-trifluoromethoxybenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-trifluoromethoxybenzoyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 595.1 (M-H).

Compound 31

(S)-3-Benzyloxy-2-[3-[3-(4-chlorophenoxy)phenyl]-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

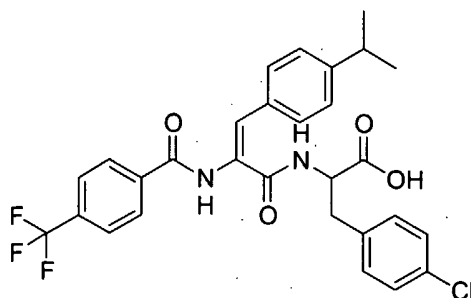
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 3-(4-chlorophenoxy)benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 639.2 (M+H)⁺.

Compound 32

(S)-3-Benzyloxy-2-[3-(4-isopropylphenyl)-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-isopropylbenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 553.3 (M-H)⁻.

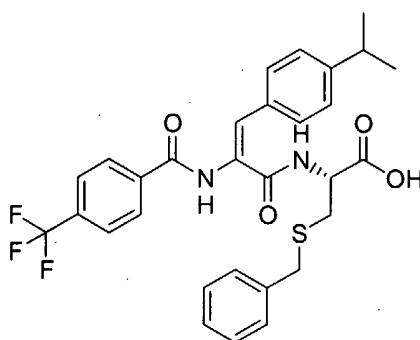
Compound 33



3-(4-Chlorophenyl)-2-[3-(4-isopropylphenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

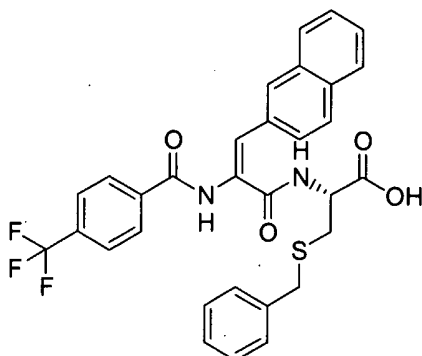
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-isopropylbenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting D,L-4-chlorophenyl-alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 557.2 (M-H)⁺.

Compound 34



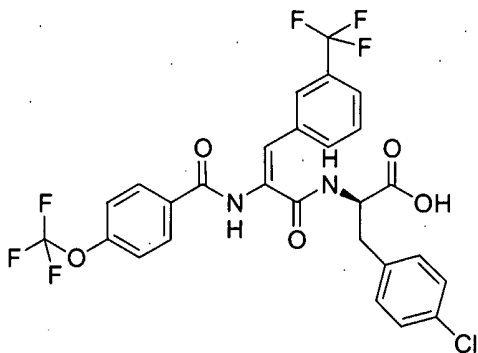
(R)-3-Benzylsulfanyl-2-[3-(4-isopropylphenyl)-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-isopropylbenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 569.3 (M-H)⁺.

Compound 35

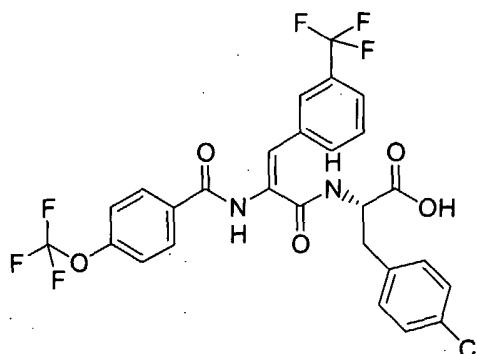
(R)-3-Benzylsulfanyl-2-[3-naphthalen-2-yl-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting naphthalene-2-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 577.2 (M-H)⁻.

Compound 36

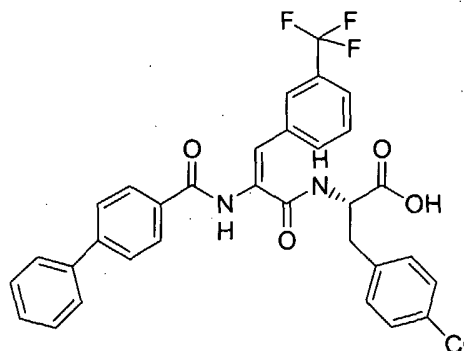
(R)-3-(4-Chlorophenyl)-2-[2-(4-trifluoromethoxybenzoylamino)-3-(3-trifluoromethylphenyl)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-trifluoromethoxybenzoyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 599.2 (M-H)⁻.

Compound 37

(S)-3-(4-Chlorophenyl)-2-[2-(4-trifluoromethoxybenzoylamino)-3-(3-trifluoromethylphenyl)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-trifluoromethoxybenzoyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 599.1 (M-H).

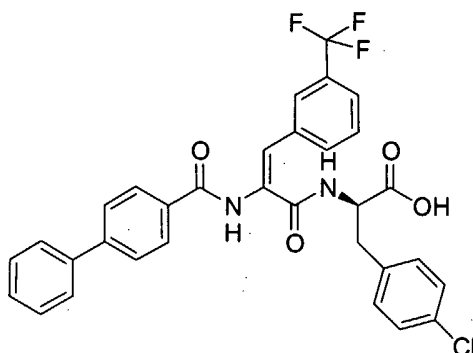
Compound 38

(S)-2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-

chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 591.2 (M-H).

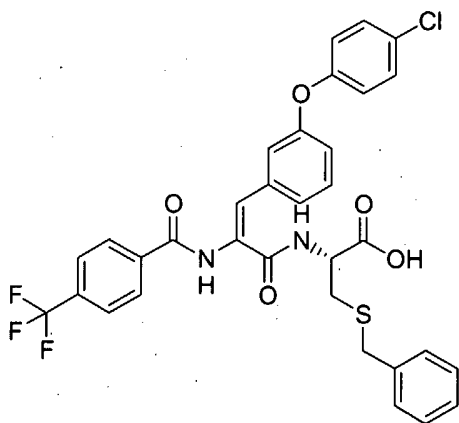
Compound 39



(R)-2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 591.2 (M-H).

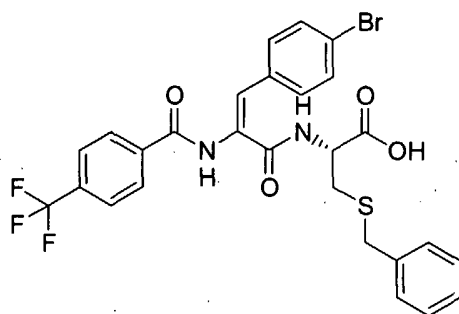
Compound 40



(R)-3-Benzylsulfanyl-2-[3-[3-(4-chlorophenoxy)phenyl]-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 3-(4-chlorophenoxy)benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 653.0 (M-H).

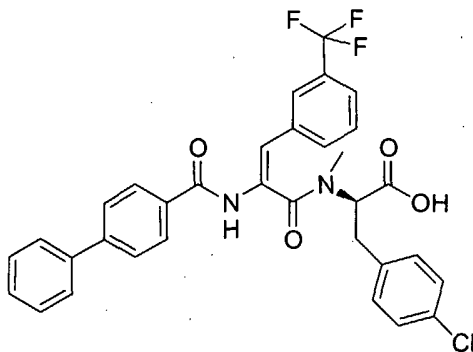
Compound 41



(R)-3-Benzylsulfanyl-2-[3-(4-bromophenyl)-2-(4-trifluoromethylbenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-bromobenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 605.0 (M-H).

Compound 42



(R)-2-{[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloyl]-methylamino}-3-(4-chlorophenyl)propionic acid

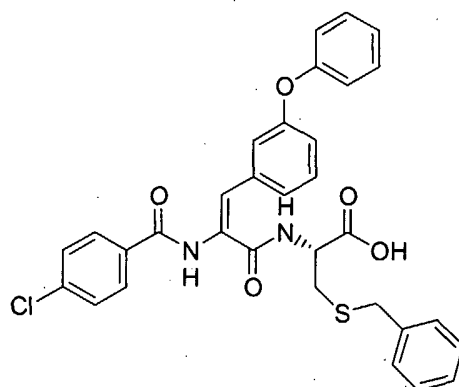
Step A: 3-(4-Chlorophenyl)-2-methylaminopropionic acid

(2R)-2-(tert-Butoxycarbonyl-methylamino)-3-(4-chlorophenyl)propionic acid (120 mg, 0.382 mmol) was put into 2N HCl in ether (1.9 ml, 3.82 mmol). The mixture was stirred at room temperature for 2 hours. White precipitate formed. The suspension was concentrated on the rotovap to give the title compound as a white solid (81 mg, 0.38 mmol).

Step B: (R)-2-{{2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)-acryloyl}methylamino}-3-(4-chlorophenyl)propionic acid

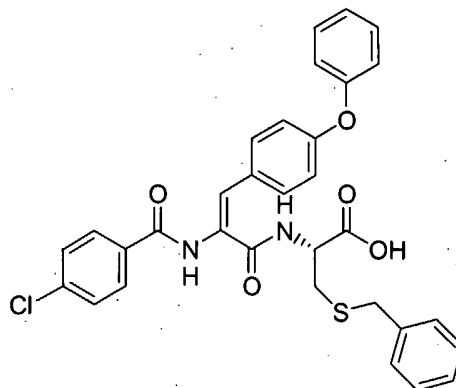
(2R)-3-(4-Chlorophenyl)-2-methylaminopropionic acid from Step A (27 mg, 0.13 mmol) was dissolved in an aqueous solution of 1N lithium hydroxide (0.27 ml, 0.27 mmol). To this solution was added 1 mL of THF followed by addition of 2-biphenyl-4-yl-4-(3-trifluoromethyl-benzylidene)-4*H*-oxazol-5-one (54 mg, 0.137 mmol, prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1). The reaction was stirred at room temperature for 2 hours and then concentrated in vacuo. The solid obtained was partitioned between EtOAc and acidic water (pH 1). The organic layer was then separated and dried over MgSO₄. After filtration the filtrate was concentrated in vacuo. Purification by HPLC gave 5 mg of the title compound as a white solid.

Compound 43



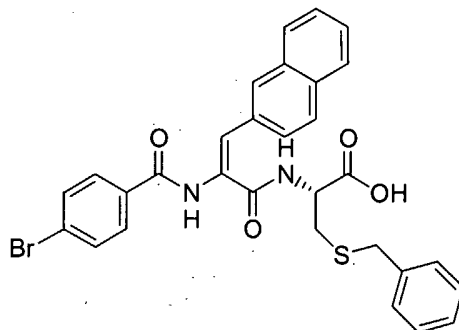
(R)-3-Benzylsulfanyl-2-[2-(4-chlorobenzoylamino)-3-(3-phenoxyphenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-chlorobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-phenoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 587.1 (M+H)⁺.

Compound 44

(R)-3-Benzylsulfanyl-2-[2-(4-chlorobenzoylamino)-3-(4-phenoxyphenyl)-acryloylamino]propionic acid

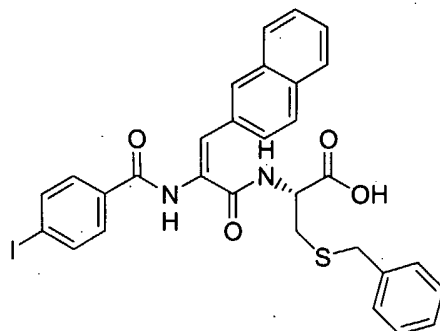
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-chlorobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 4-phenoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 585.2 (M+H)⁺.

Compound 45

(R)-3-Benzylsulfanyl-2-[2-(4-bromobenzoylamino)-3-naphthalen-2-yl-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-bromobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting naphthalene-2-carboxaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 589.0 (M+H)⁺.

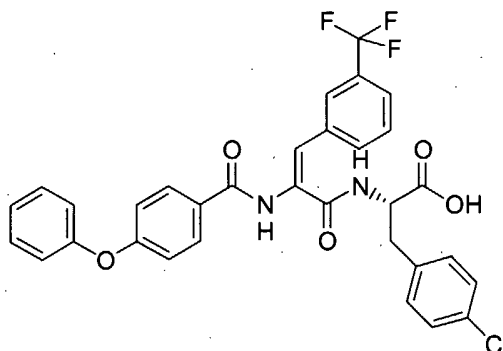
Compound 46



(R)-3-Benzylsulfanyl-2-[2-(4-iodobenzoylamino)-3-naphthalen-2-yl-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-iodobenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting naphthalene-2-carboxaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 635.0 (M+H)⁺.

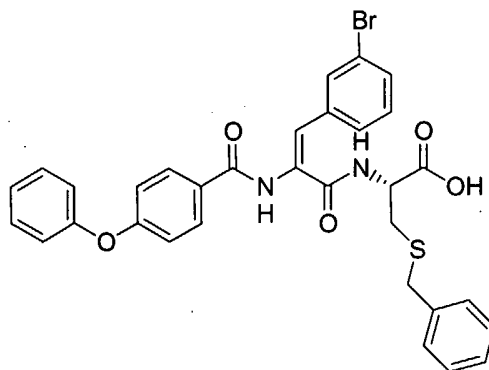
Compound 47



(S)-3-(4-Chlorophenyl)-2-[2-(4-phenoxybenzoylamino)-3-(3-trifluoromethyl-phenyl)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 607.0 (M-H)⁻.

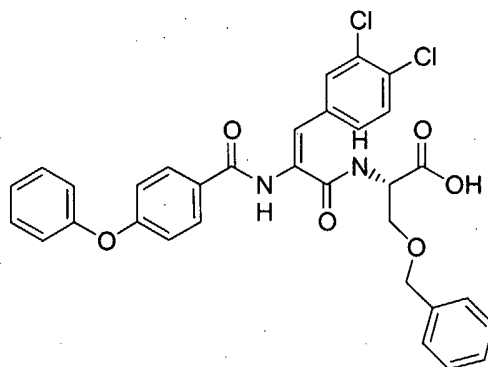
Compound 48



(R)-3-Benzylsulfanyl-2-[3-(3-bromophenyl)-2-(4-phenoxybenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-bromo-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 631.0 (M-H)⁺.

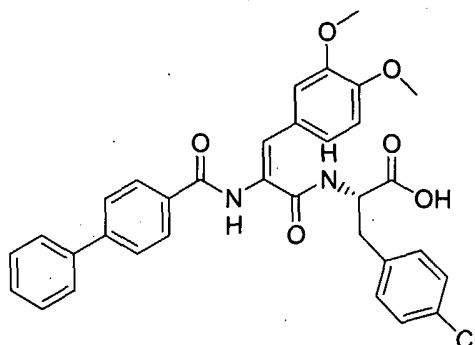
Compound 49



(S)-3-Benzylloxy-2-[3-(3,4-dichlorophenyl)-2-(4-phenoxybenzoylamino)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-phenoxybenzoyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichloro-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-L-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 603.2 (M-H)⁺.

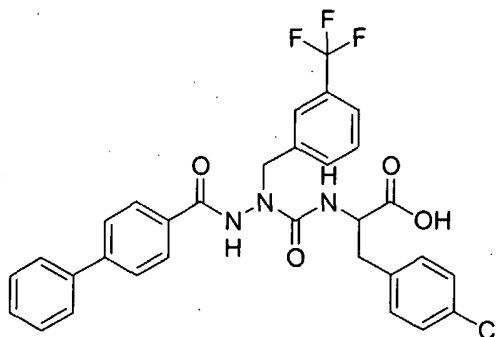
Compound 50



2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3,4-dimethoxyphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

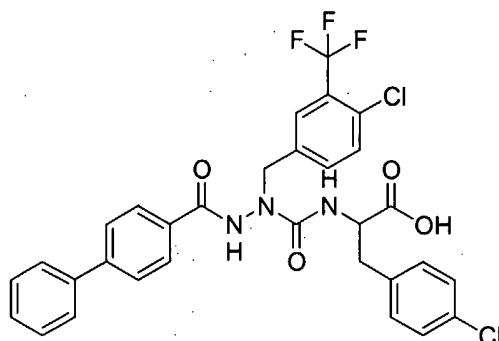
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3,4-dimethoxy-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 583.2 (M-H)⁻.

Compound 51



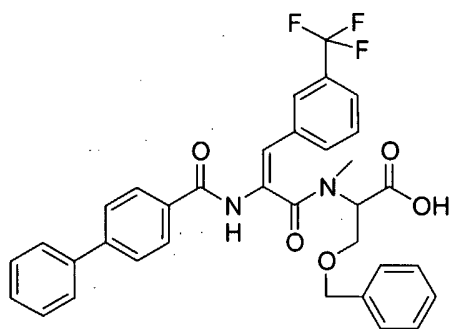
4-Biphenylcarboxylic acid-N'-[(3-trifluoromethylphenyl)methyl]-N'-[[[2-(4-chlorophenyl)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting biphenyl-4-carboxylic acid hydrazide for the 4-trifluoromethylbenzoic acid hydrazide, and 3-trifluoromethyl-benzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8; and by substituting D,L-4-chlorophenylalanine ethyl ester hydrochloride for the S-benzyl-L-cysteine methyl ester hydrochloride of Step C of Compound 8. MS 594.1 (M-H)⁻.

Compound 52

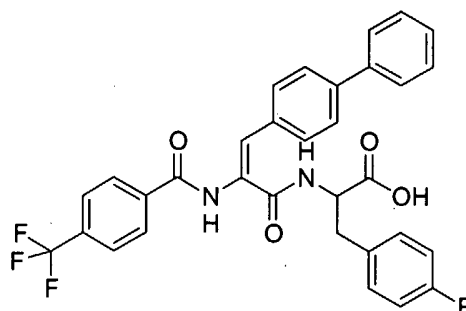
4-Biphenylcarboxylic acid-N'-[(4-chloro-3-trifluoromethylphenyl)methyl]-N'-[[[2-(4-chlorophenyl)-1-carboxyethyl]amino]carbonyl]hydrazide

The title compound was prepared by a procedure analogous to that of Compound 8 by substituting biphenyl-4-carboxylic acid hydrazide for the 4-trifluoromethylbenzoic acid hydrazide, and 3-trifluoromethyl-4-chloro-benzaldehyde for the biphenyl-4-carboxaldehyde of Step A of Compound 8; and by substituting D,L-4-chlorophenylalanine ethyl ester hydrochloride for the S-benzyl-L-cysteine methyl ester hydrochloride of Step C of Compound 8. MS 594.1 (M-H)⁺.

Compound 53

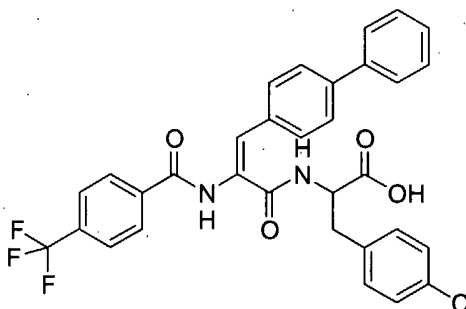
3-Benzyloxy-2-{{[2-[(biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloyl]methylamino}propionic acid

The title compound was prepared by a procedure analogous to that of Compound 42 by substituting 3-benzyloxy-2-(tert-butoxycarbonyl-methylamino)-propionic acid for the (2R)-2-(tert-butoxycarbonyl-methylamino)-3-(4-chloro-phenyl)propionic acid of Step A of Compound 42. MS 601.2 (M-H)⁺.

Compound 54

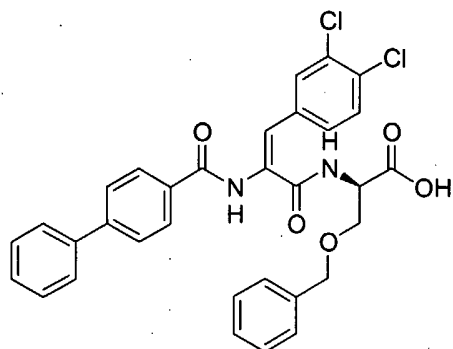
2-[3-Biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)acryloylamino]-3-(4-fluorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting D,L-4-fluorophenyl-alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 575.2 (M-H)⁺.

Compound 55

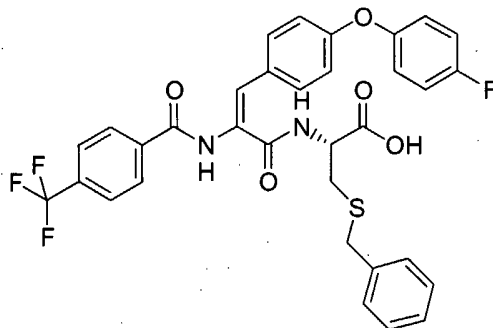
2-[3-Biphenyl-4-yl-2-(4-trifluoromethylbenzoylamino)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carboxaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting D,L-4-chlorophenyl-alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 591.1 (M-H)⁺.

Compound 56

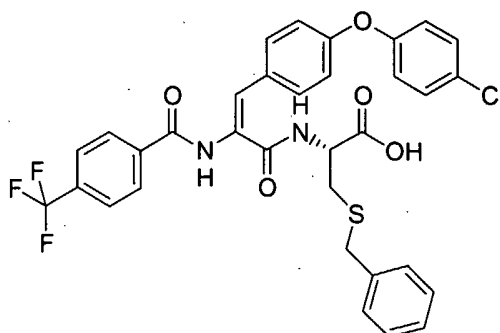
(R)-3-Benzoyloxy-2-[2-[(biphenyl-4-carbonyl)amino]-3-(3,4-dichlorophenyl)-acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichloro-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 583.2 (M-H)⁻.

Compound 57

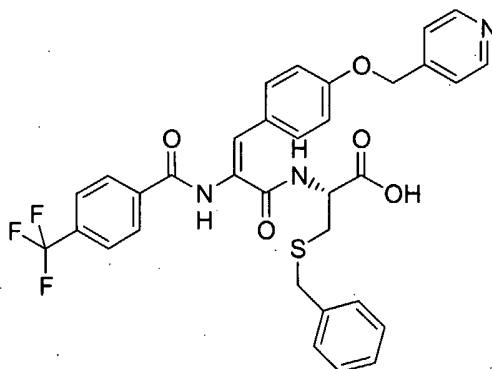
(R)-3-Benzylsulfanyl-2-[3-[4-(4-fluorophenoxy)phenyl]-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-(4-fluorophenoxy)benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 637.0 (M-H)⁻.

Compound 58

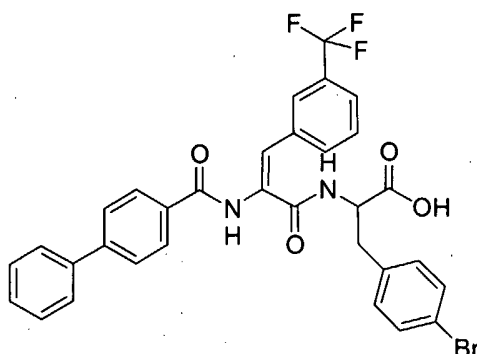
(R)-3-Benzylsulfanyl-2-[3-[4-(4-chlorophenoxy)phenyl]-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-(4-chlorophenoxy)benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 655.0 (M+H)⁺.

Compound 59

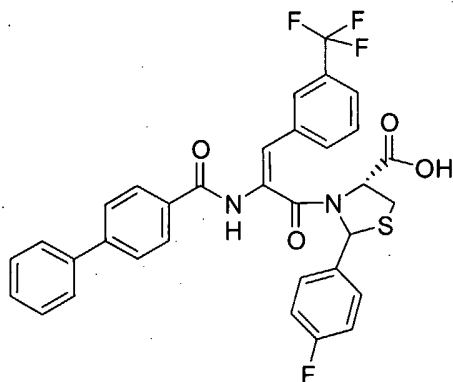
(R)-3-Benzylsulfanyl-2-[3-[4-(pyridin-4-yl-methoxy)phenyl]-2-(4-trifluoromethylbenzoylamino)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 4-(pyridin-4-yl-methoxy)benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 636.2 (M+H)⁺.

Compound 60

2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-bromophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D,L-4-bromophenyl alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 635.0 (M-H)⁺.

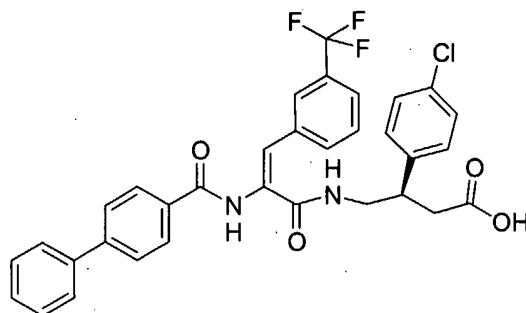
Compound 61

(4R)-3-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloyl]-2-(4-fluorophenyl)thiazolidine-4-carboxylic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting 2-(4-

fluorophenyl)thiazolidine-4-carboxylic acid for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 643.0 (M+Na)⁺.

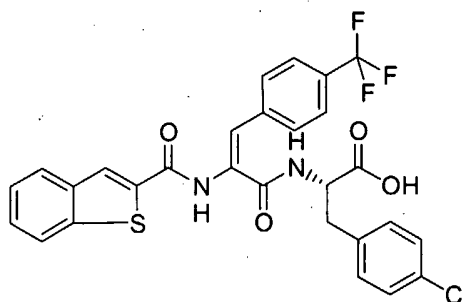
Compound 62



(R)-4-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)butyric acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting (R)-4-amino-3-(4-chlorophenyl)butyric acid for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 605.1 (M-H)⁻.

Compound 63

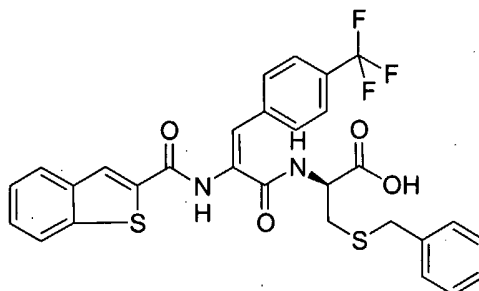


(S)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(4-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-

chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 571.0 (M-H)⁺.

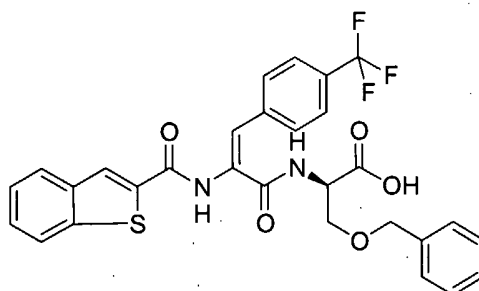
Compound 64



(S)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(4-trifluoromethylphenyl)-acryloylamino]-3-benzylsulfanylpropionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Cys(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 583.0 (M-H)⁺.

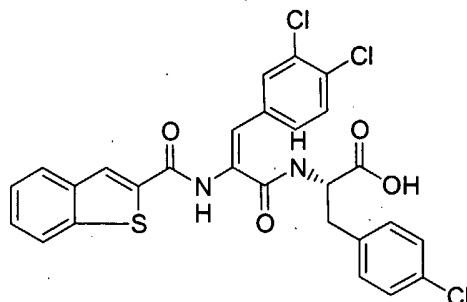
Compound 65



(R)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(4-trifluoromethylphenyl)-acryloylamino]-3-benzyloxypropionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 567.0 (M-H)⁺.

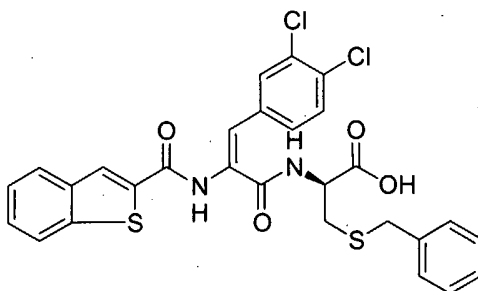
Compound 66



(S)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(3,4-dichlorophenyl)-acryloylamino]-3-(4-chlorophenyl)propionic acid

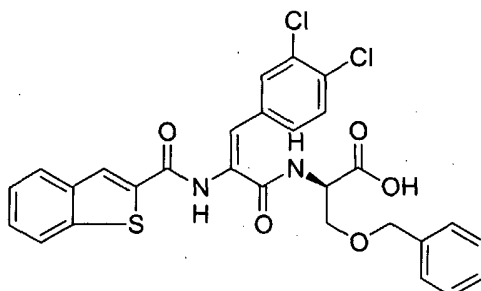
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 573.0 (M-H)⁺.

Compound 67



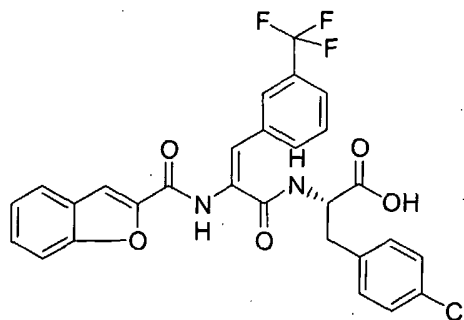
(S)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(3,4-dichlorophenyl)-acryloylamino]-3-benzylsulfanylpropionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Cys(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 583.0 (M-H)⁺.

Compound 68

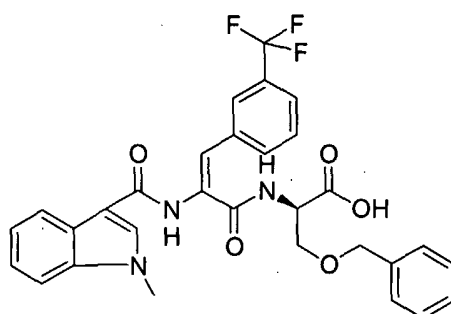
(R)-2-[2-[(Benzo[b]thiophene-2-carbonyl)amino]-3-(3,4-dichlorophenyl)-acryloylamino]-3-benzyloxypropionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]thiophene-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 567.0 (M-H)⁻.

Compound 69

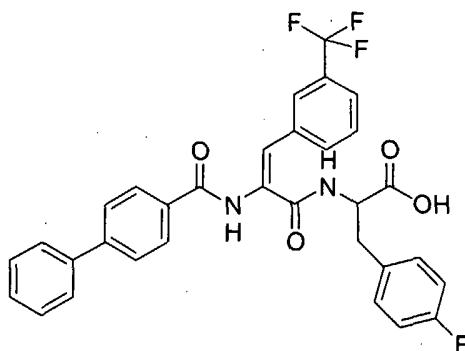
(S)-2-[2-[(Benzofuran-2-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting benzo[b]furan-2-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 557.1 (M-H)⁻.

Compound 70

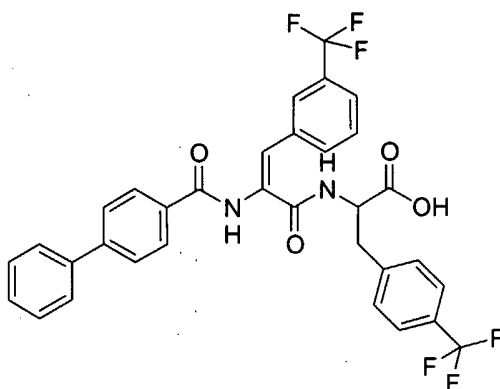
(R)-3-Benzyloxy-2-[2-[(1-methyl-1H-indole-3-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-methyl-1H-indole-3-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 564.2 (M-H)⁻.

Compound 71

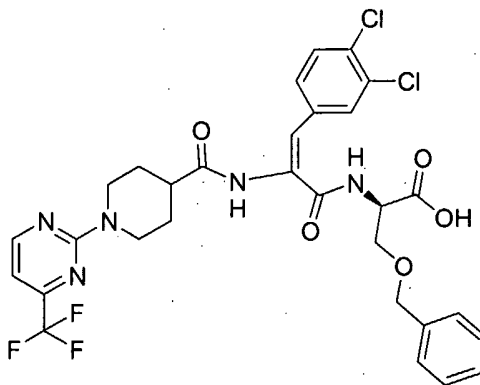
2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-fluorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D,L-4-fluorophenyl alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 577.0 (M+H)⁺.

Compound 72

2-[2-[(Biphenyl-4-carbonyl)amino]-3-(3-trifluoromethylphenyl)acryloylamino]-3-(4-trifluoromethylphenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting biphenyl-4-carbonyl chloride for the 4-trifluoromethyl-benzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl-benzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting D,L-4-trifluoromethylphenyl alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 627.0 (M+H)⁺.

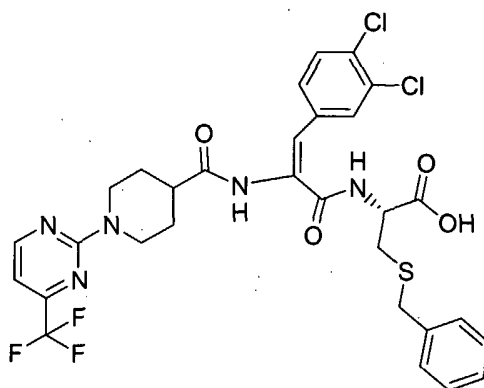
Compound 73

(R)-3-Benzyloxy-2-(3-(3,4-dichlorophenyl)-2-{[1-(4-trifluoromethylpyrimidin-2-yl)-piperidine-4-carbonyl]amino}acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and

by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 664.0 (M-H)⁺.

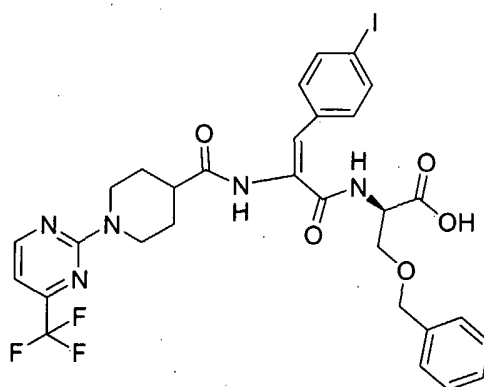
Compound 74



(R)-3-Benzylsulfanyl-2-(3-(3,4-dichlorophenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3,4-dichlorobenzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 680.0 (M-H)⁺.

Compound 75

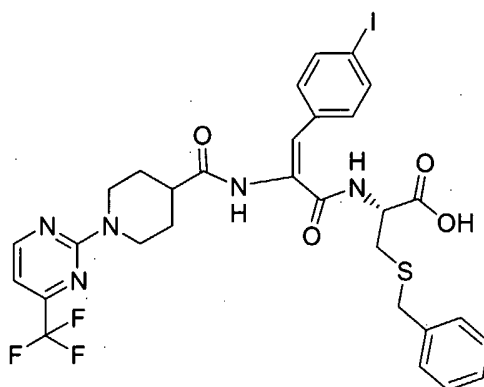


(R)-3-Benzoyloxy-2-(3-(4-iodophenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-

iodobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 773.9 (M+H)⁺.

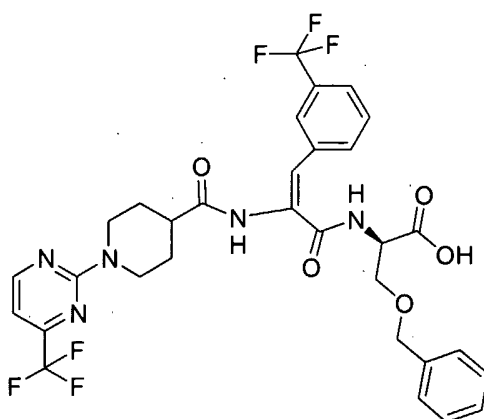
Compound 76



(R)-3-Benzylsulfanyl-2-(3-(4-iodophenyl)-2-{[1-(4-trifluoromethylpyrimidin-2-yl)-piperidine-4-carbonyl]amino}acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-iodobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 738.0 (M-H)⁻.

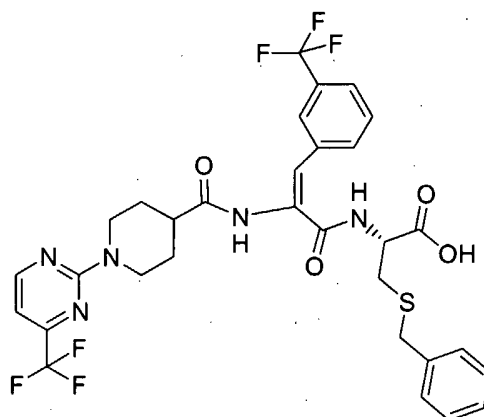
Compound 77



(R)-3-Benzylsulfanyl-2-(3-(3-trifluoromethylphenyl)-2-{[1-(4-trifluoromethylpyrimidin-2-yl)-piperidine-4-carbonyl]amino}acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 666.0 (M+H)⁺.

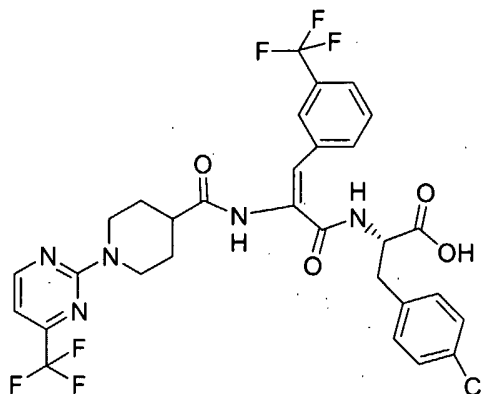
Compound 78



(R)-3-Benzylsulfanyl-2-(3-(3-trifluoromethylphenyl)-2-{[1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino}acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1. MS 682.0 (M+H)⁺.

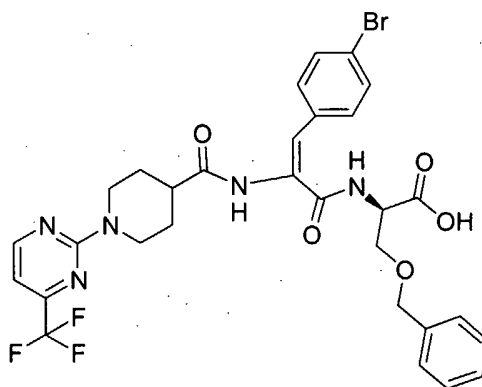
Compound 79



(S)-3-(4-Chlorophenyl)-2-(3-(3-trifluoromethylphenyl)-2-{{[1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino}acryloylamino)propionic acid

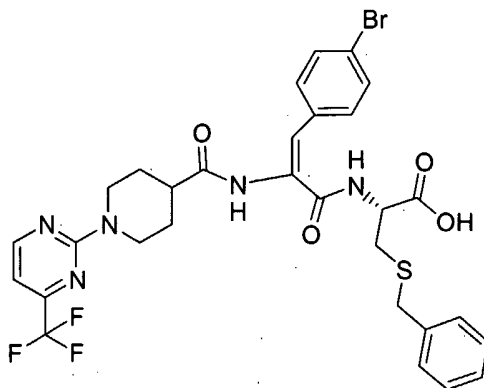
The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 3-trifluoromethyl benzaldehyde for the 4-benzyloxy-benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenyl-alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 670.0 (M+H)⁺.

Compound 80



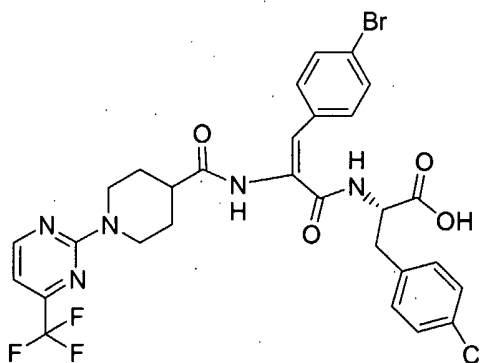
(R)-3-Benzyloxy-2-(3-(4-bromophenyl)-2-{{[1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino}acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-bromobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 674.1 (M-H)⁻.

Compound 81

(R)-3-Benzylsulfanyl-2-(3-(4-bromophenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-bromobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 692.0 (M+H)⁺.

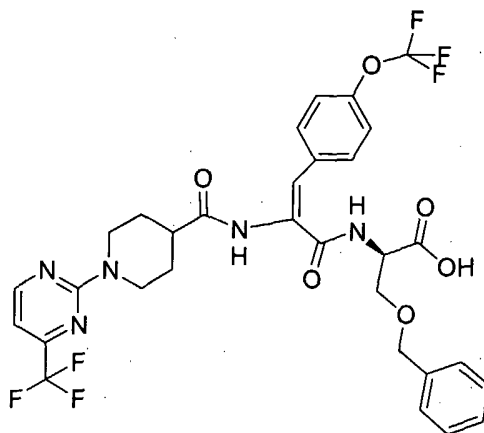
Compound 82

(S)-2-(3-(4-Bromophenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)-3-(4-chlorophenyl)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-bromobenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by

substituting L-4-chlorophenylalanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 681.9 (M+H)⁺.

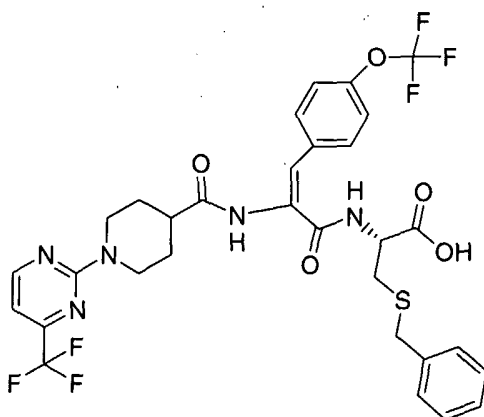
Compound 83



(R)-3-Benzyloxy-2-(3-(4-trifluoromethoxyphenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethoxybenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting H-Ser(Bzl)-D-OH for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 682.0 (M+H)⁺.

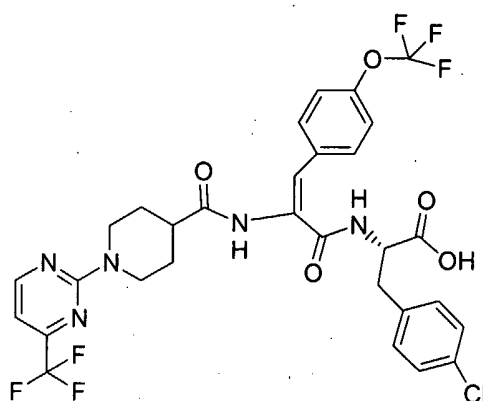
Compound 84



(R)-3-Benzylsulfanyl-2-(3-(4-trifluoromethoxyphenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethoxybenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1. MS 698.0 (M+H)⁺.

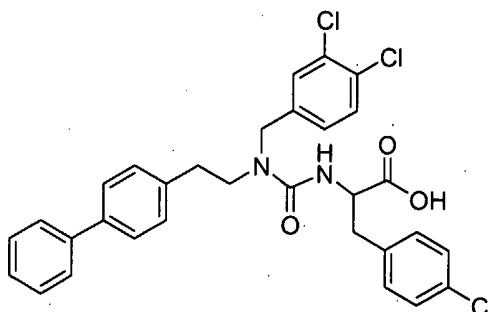
Compound 85



(S)-3-(4-Chlorophenyl)-2-(3-(4-trifluoromethoxyphenyl)-2-([1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl]amino)acryloylamino)propionic acid

The title compound was prepared by a procedure analogous to that of Compound 1 by substituting 1-(4-trifluoromethylpyrimidin-2-yl)piperidine-4-carbonyl chloride for the 4-trifluoromethylbenzoyl chloride of Step A of Compound 1; and by substituting 4-trifluoromethoxybenzaldehyde for the 4-benzyloxy benzaldehyde of Step C of Compound 1; and by substituting L-4-chlorophenyl-alanine for the H-Cys(Bzl)-L-OH of Step D of Compound 1. MS 687.0 (M+H)⁺.

Compound 86



2-[3-(2-Biphenyl-4-yl-ethyl)-3-(3,4-dichlorobenzyl)ureido]-3-(4-chlorophenyl)-propionic acid

Step A: N-(2-Biphenyl-4-yl-ethyl)-3,4-dichloro-benzylamine

2-Biphenyl-4-yl-ethylamine (0.3 g, 1.52 mmol) and 3,4-dichloro-benzaldehyde (0.266 g, 1.52 mmol) were dissolved in ethanol (3 ml) at room temperature under nitrogen atmosphere and the resulting reaction mixture was heated at 70 °C for 2 hours. It was then cooled to room temperature and sodium triacetoxyborohydride (0.322 g, 1.52 mmol) was added. After 16 h the reaction mixture was concentrated in vacuum. The crude product was dissolved in EtOAc, and washed with saturated NH₄Cl (aq.), water and brine. The organic layer was dried over MgSO₄, filtered and concentrated in vacuo. Purification by medium pressure liquid chromatography on silica gel (3:100 methanol/dichloromethane) gave 286 mg (53%) of the title compound as a colorless oil. MS 356.1 (M+H)⁺.

Step B: 2-[3-(2-Biphenyl-4-yl-ethyl)-3-(3,4-dichlorobenzyl)ureido]-3-(4-chlorophenyl)propionic acid ethyl ester

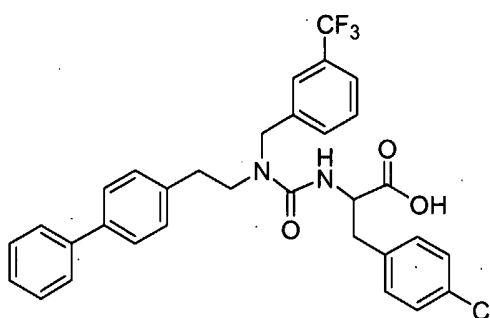
To a mixture of D,L-4-chlorophenylalanine ethyl ester hydrochloride (148 mg, 0.56 mmol) and 1,1'-carbonyldiimidazole (100 mg, 0.614 mmol) in anhydrous dichloromethane (4 ml) at room temperature under nitrogen atmosphere, was added triethylamine (0.16 ml, 114 mg, 1.12 mmol). After 10 minutes, N-(2-biphenyl-4-yl-ethyl)-3,4-dichlorobenzylamine from Step A (200 mg, 0.56 mmol) was added and the reaction mixture was stirred for 16 h. The reaction mixture was then washed with NaOH (1 N, 2 x 5 ml). The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo. Purification of the crude product by medium pressure liquid chromatography on silica gel (20:80 ethyl acetate/hexane) gave 225 mg (66%) of the title compound as a white solid. MS 611.2 (M+H)⁺.

Step C: 2-[3-(2-Biphenyl-4-yl-ethyl)-3-(3,4-dichlorobenzyl)ureido]-3-(4-chlorophenyl)propionic acid

To solution of 2-[3-(2-biphenyl-4-yl-ethyl)-3-(3,4-dichlorobenzyl)ureido]-3-(4-chlorophenyl)propionic acid ethyl ester from Step B (61 mg, 0.1 mmol) in THF (3 ml) was added 1N NaOH (0.5 ml) and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 16 h. After the organic solvent was removed in vacuo, the resulting aqueous solution was acidified with 1N HCl to pH 3 and extracted with EtOAc (2 x 15 ml). The combined organic layers were dried over MgSO₄, filtered, and

concentrated in vacuo. Purification of the crude product by medium pressure liquid chromatography on silica gel (5:100 methanol/dichloromethane) gave 31 mg (53%) of the title compound as a white solid. MS 581.0 (M-H)⁻.

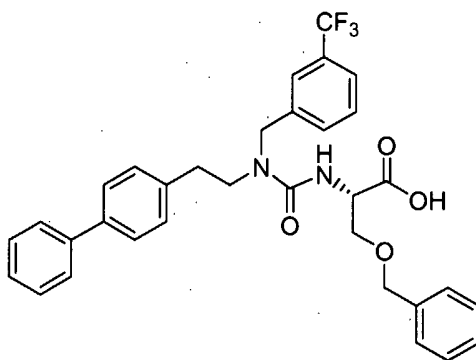
Compound 87



2-[3-(2-Biphenyl-4-yl-ethyl)-3-(3-trifluoromethylbenzyl)ureido]-3-(4-chlorophenyl)propionic acid

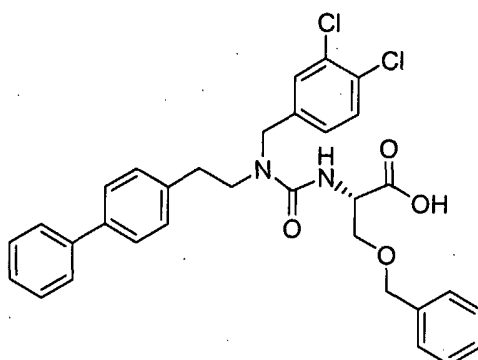
The title compound was prepared by a procedure analogous to that of Compound 86 by substituting 3-trifluoromethyl benzaldehyde for the 3,4-dichlorobenzaldehyde of Step A of Compound 86. MS 581.1 (M+H)⁺.

Compound 88



(S)-3-Benzyloxy-2-[3-(2-biphenyl-4-yl-ethyl)-3-(3-trifluoromethylbenzyl)ureido]propionic acid

The title compound was prepared by a procedure analogous to that of Compound 86 by substituting 3-trifluoromethyl benzaldehyde for the 3,4-dichloro-benzaldehyde of Step A of Compound 86, and by substituting H-Ser(Bzl)-L-OMe hydrochloride for the D,L-4-chlorophenylalanine ethyl ester hydrochloride of Step B of Compound 86. MS 577.1 (M+H)⁺.

Compound 89

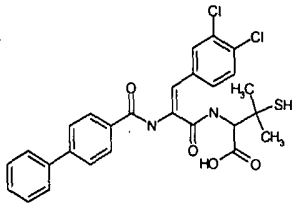
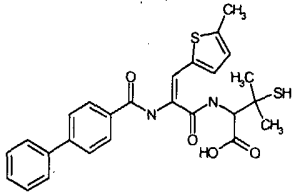
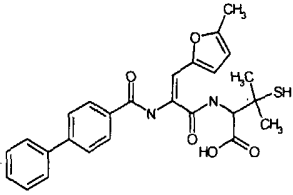
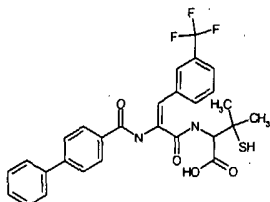
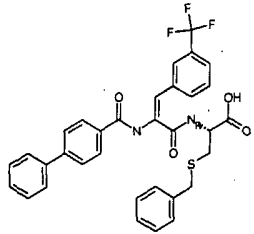
(S)-3-Benzyloxy-2-[3-(2-biphenyl-4-yl-ethyl)-3-(3,4-dichlorobenzyl)ureido]propionic acid

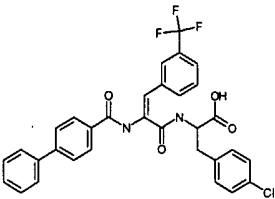
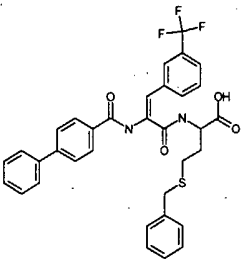
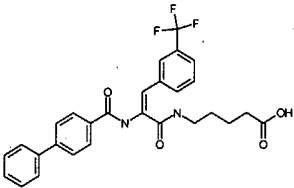
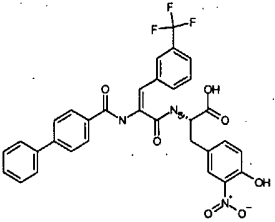
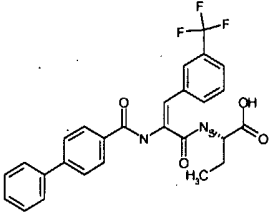
The title compound was prepared by a procedure analogous to that of Compound 86 by substituting H-Ser(Bzl)-L-OMe hydrochloride for the D,L-4-chlorophenylalanine ethyl ester hydrochloride of Step B of Compound 86. MS 575.1 (M-H)⁺.

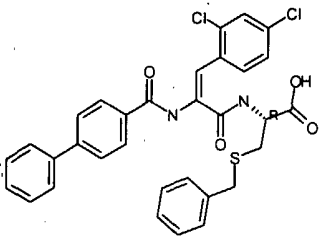
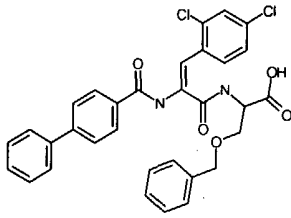
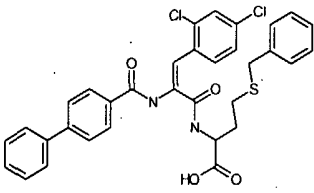
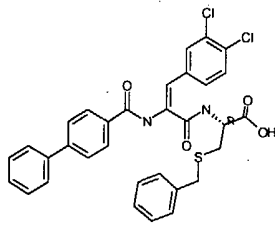
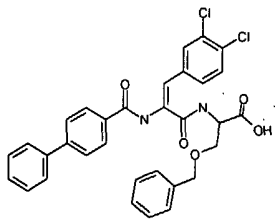
The following compounds were prepared by methods analogous to those described in Compound 1:

Table I

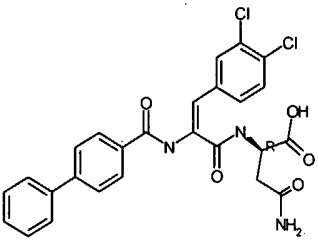
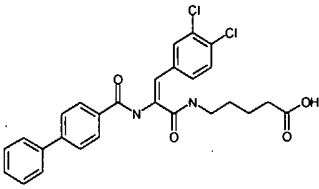
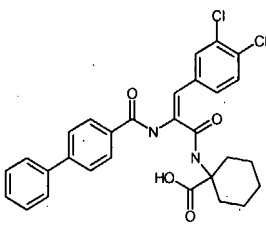
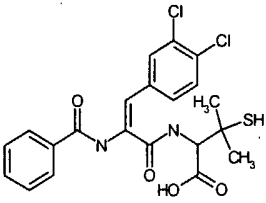
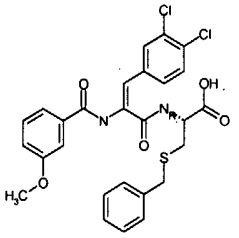
Compound	Structure	Molecular Weight	[M+1]	[M-1]
90		542.576	543.21	
91		585.791	585.01	

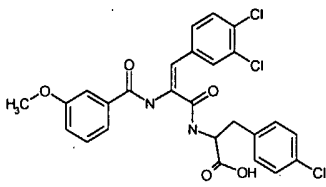
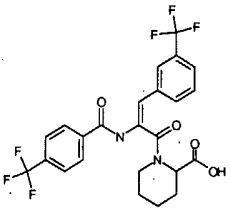
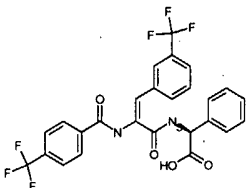
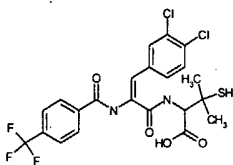
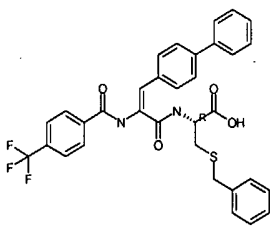
Compound	Structure	Molecular Weight	[M+1]	[M-1]
92		543.469	543.06	
93		494.633	494.24	493.55
94		478.566	479.17	
95		542.576	542.91	
96		604.646	604.9	

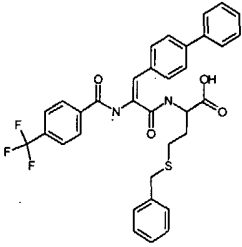
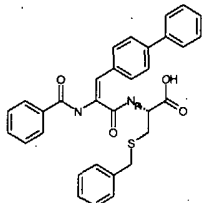
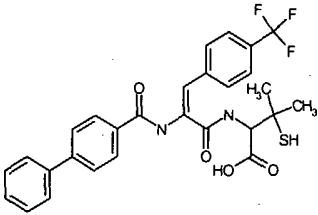
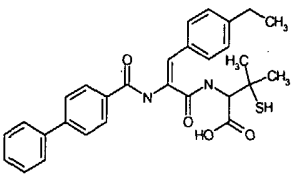
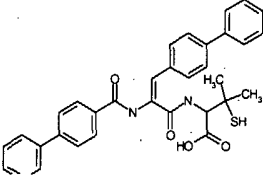
Compound	Structure	Molecular Weight	[M+1]	[M-1]
97		592.999	592.81	
98		618.673	619.04	
99		510.509	511.07	
100		619.55	620.1	
101		496.483		495.23

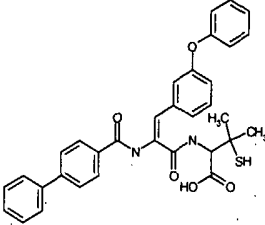
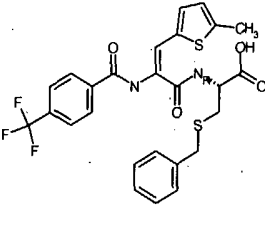
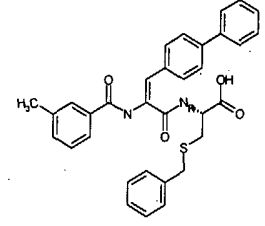
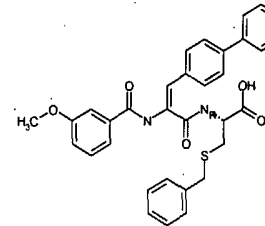
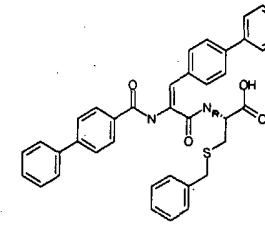
Compound	Structure	Molecular Weight	[M+1]	[M-1]
102		605.539	605.03	
103		589.472	590.85	
104		619.566	619.08	
105		605.539	605.1	
106		589.472	589.06	

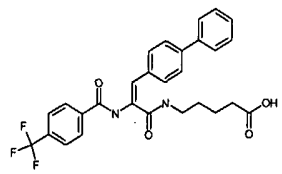
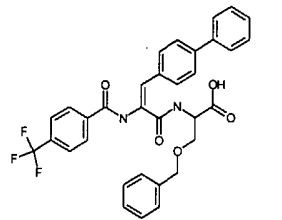
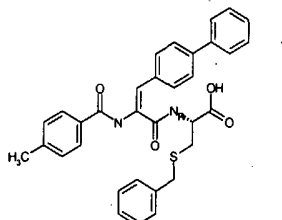
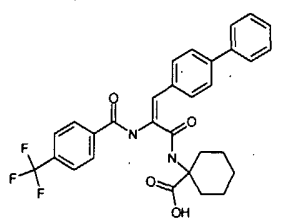
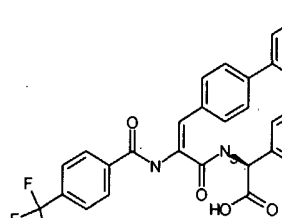
Compound	Structure	Molecular Weight	[M+1]	[M-1]
107		523.414	523.01	
108		575.467	575.01	
109		620.443		618.13
110		545.42	545.08	
111		513.375	513.02	

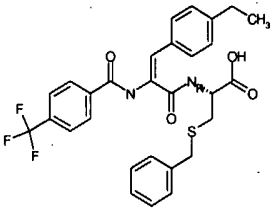
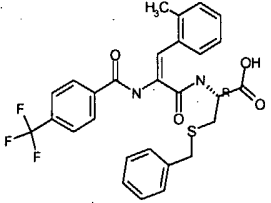
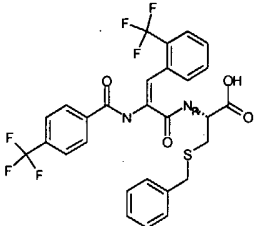
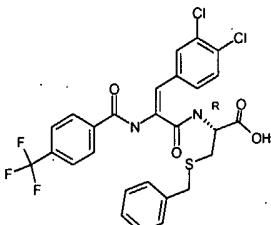
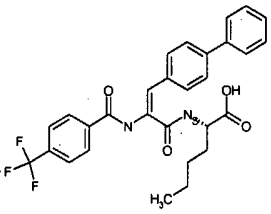
Compound	Structure	Molecular Weight	[M+1]	[M-1]
112		526.374	526.23	
113		511.403	511.13	
114		537.44	537.16	
115		467.371	467.21	
116		559.468		559.3

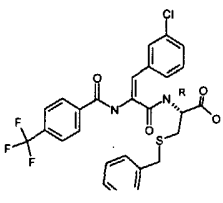
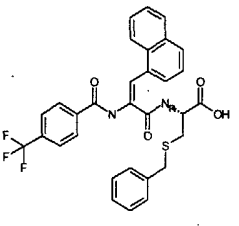
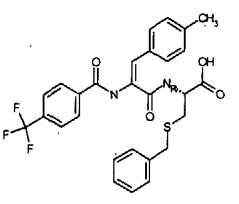
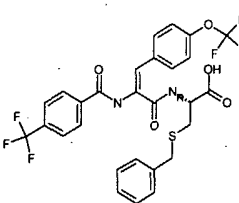
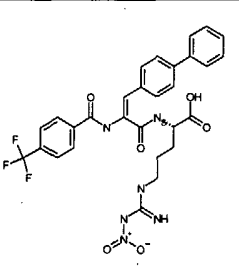
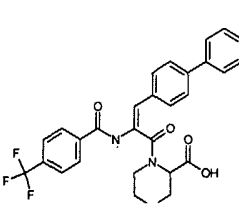
Compound	Structure	Molecular Weight	[M+1]	[M-1]
117		547.82	548.88	
118		514.42	515.21	
119		536.426	537.19	
120		535.368	535.18	
121		604.646	605.1	

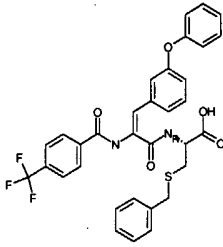
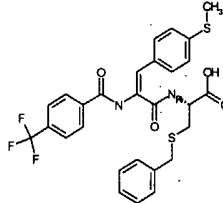
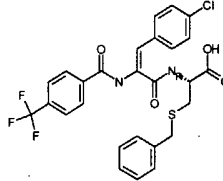
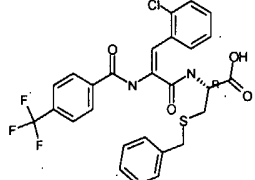
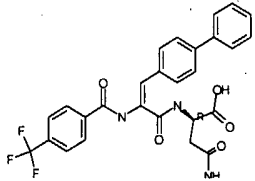
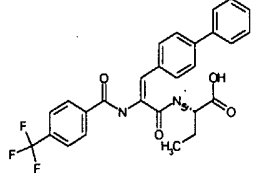
Compound	Structure	Molecular Weight	[M+1]	[M-1]
122		618.673	619.34	
123		536.649		535.35
124		542.576		541.32
125		502.632		501.36
126		550.676		549.37

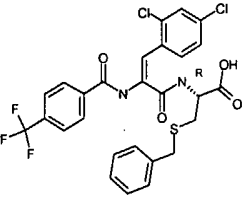
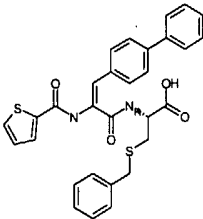
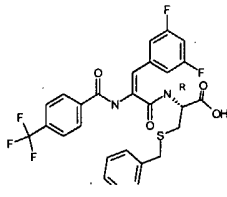
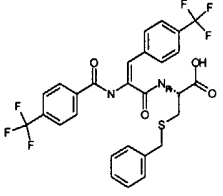
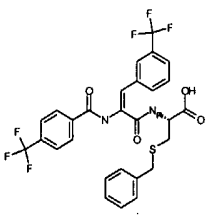
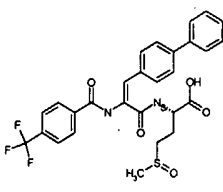
Compound	Structure	Molecular Weight	[M+1]	[M-1]
127		566.675		565.32
128		548.604		547.28
129		550.676		549.37
130		566.675		565.42
131		612.747		611.48

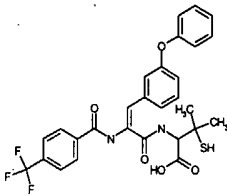
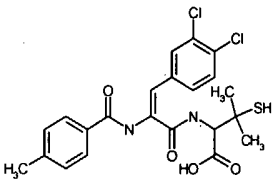
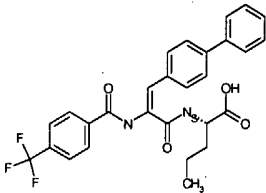
Compound	Structure	Molecular Weight	[M+1]	[M-1]
132		510.509	511.46	
133		588.579		587.4
134		550.676		549.38
135		536.547		535.4
136		544.527		543.29

Compound	Structure	Molecular Weight	[M+1]	[M-1]
137		556.602		555.4
138		542.576		541.38
139		596.546		595.3
140		597.439		595.19
141		524.536		523.39

Compound	Structure	Molecular Weight	[M+1]	[M-1]
142		562.994		561.31
143		578.609		577.36
144		542.576		541.38
145		612.545		611.34
146		612.562		611.36
147		522.521		521.34

Compound	Structure	Molecular Weight	[M+1]	[M-1]
148		620.645		619.33
149		574.641		573.26
150		562.994		561.31
151		562.994		561.3
152		525.481		524.35
153		496.483		495.31

Compound	Structure	Molecular Weight	[M+1]	[M-1]
154		597.439		595.14
155		542.677	543.13	
156		564.529		563.21
157		596.546		595.38
158		596.546		595.34
159		558.575		557.35

Compound	Structure	Molecular Weight	[M+1]	[M-1]
160		558.575		557.36
161		481.398		479.28
162		510.509		509.37

Example 2: Assay to Evaluate Effect on Type III Protein Secretion Systems

The ability of the compounds of the invention to inhibit Type III protein secretion systems may be analyzed as follows.

Primary assay: Type III protein secretion of the chimeric SopE'-Bla polypeptide by *Salmonella enterica*. This procedure is a cell-based assay that measures the type III-dependent secretion by *Salmonella enterica* of a plasmid-encoded chimeric polypeptide whose synthesis can be regulated, and which is endowed with an enzymatic activity that can be monitored colorimetrically by hydrolysis of a substrate that is unable to penetrate into the bacterial cytoplasm within the time constraints of the reaction. Thus, the colorimetric reaction is not influenced by SopE'-Bla polypeptide in the bacterial cytoplasm. Instead, it effectively measures the amount of polypeptide that has been secreted from the *S. enterica* cytoplasm to the extracellular medium via type III system protein secretion.

The SopE'-Bla recombinant polypeptide consists of two functionally distinct domains spliced together. The N-terminus domain is encoded by a polynucleotide region specifying the signal for type III secretion of the SopE polypeptide of *S. enterica*, an effector of the SPI1 type III protein secretion system. The C-terminus domain of SopE'-Bla consists of a 263 amino acid peptide sequence that corresponds to the TEM-1 β -lactamase expressed by plasmid pBR322 but without its N-terminal signal sequence. The TEM-1 β -lactamase part of the SopE'-Bla chimeric polypeptide is used as a reporter enzyme. It is capable of hydrolyzing nitrocefin resulting in a product whose accumulation can be monitored by colorimetric detection. The secretion of the SopE'-Bla chimeric polypeptide from the cytoplasm to the extracellular medium is dependent on type III protein secretion.

For this procedure, cells grown under conditions known to favor a functional SPI1 secretion system are induced for expression of the SopE'-Bla protein and grown either in the presence or in the absence of putative inhibitors for determined time. Nitrocefin is then added to the various cultures and its hydrolysis are used for quantitation. An inhibitor of Type III protein secretion is generally a compound that reduces the signal of the enzymatic reaction by decreasing the amount of SopE'-Bla secreted into the extracellular medium.

Secondary assay: Type III-dependent protein secretion of the SipB polypeptide by *S. enterica*. The SipB protein of *S. enterica* is another effector of the SPI1 type III protein secretion system from *S. enterica*. In this cell-based procedure, the Type III-dependent secretion of SipB from the bacterial cytoplasm to the extracellular medium was measured through its reactivity with a cognate mouse monoclonal.

Salmonella enterica cells growing either in the presence or in the absence of inhibitors are induced for the production of SipB. Following an established period of growth the cells are sedimented and the amount of SipB present in the supernatant is quantified with a scanning imager following application of immunoblot techniques. Detection may employ an anti-SipB mouse monoclonal antibody (e.g., obtained from Jorge Galan, SUNY at Stony Brook, NY) followed by treatment with commercially available sheep anti-mouse polyclonal antibody conjugated with horseradish peroxidase. Thereafter the membrane is treated with a peroxidase chemiluminescent substrate and

exposed to film for an appropriate exposure time. Inhibition may be measured relative to untreated controls.

Tertiary assay: inhibition of Type III protein secretion of effectors from a *Pseudomonas aeruginosa* system. Type III protein secretion is used by *P. aeruginosa* to secrete several essential virulence determinants. One effector of the type III protein secretion system of *P. aeruginosa* PA103 is the virulence determinants ExoU.

The amount of Type III-dependent secretion of ExoU by *P. aeruginosa* PA103 can be determined in a cell-based assay by quantification of the 73.9 kDa ExoU protein secreted into the extracellular medium. Such quantitation can be achieved by growing strain PA103 in a deferrated medium in the presence of nitrilotriacetic acid (an inducer of Type III protein secretion in *P. aeruginosa*) and either in the presence or absence of putative inhibitors. After a prolonged growth period, the cells are sedimented and the supernatants concentrated by ammonium sulfate precipitation. The proteins in the resuspended pellets are separated by electrophoresis on SDS-polyacrylamide gels. After staining gels with Colloidal Blue™, the 73.9 kDa ExoU band is quantitated by scanning through an imager. The effects of inhibitors on the intensity of the ExoU band may be measured relative to that of untreated controls.

By way of example, assay results for preferred compounds of the invention are provided below in Table II.

Table II

Compound	ExoU IC50(μM)	SipB IC50(μM)	SopE IC50(μM)
1	11.6		
2			69.0
3			30.3
4			1.10
5			0.80
6	47.0		
7	31.0		
8			10.0
9			15.0
10			22.0
11			49.0
12			12.5
13			5.2
14		77.3	
15			11.0
16			4.9

Compound	ExoU IC50(μ M)	SipB IC50(μ M)	SopE IC50(μ M)
17	49.0		
18	57.0		
19			
20	37.0		
21	69.0		
22	47.0		
23	29.0		
24			12.5
25			6.0
26	24.0		
27			87.0
28			86.0
29			70.0
30	54.0		
31			7.7
32			34.8
33			10.2
34	50.0		
35	<12.5		
36			21.6
37			25.0
38	17.3		
39	17.0		
40	13.1		
41			48.0
42			15.0
43			34.2
44	26.5		
45			81.6
46			28.6
47	35.1		12.4
48	24.8		
49	75.7		
50			79.4
51			8.0
52			3.1
53	46.0		
54	19.0		
55	13.0		
56	20.2		
57	35.0		
58	48.5		
59			73.4
60			9.2
61			3.7
62	87.8		
63			37.4

Compound	ExoU IC50(μ M)	SipB IC50(μ M)	SopE IC50(μ M)
64			49.4
65			88.0
66			19.0
67			27.0
68			52.0
69			28.5
70			86.3
71			34.2
72			12.8
73			12.8
74	33.8		
75	66.3		
76	56.7		
77	60.0		
78	67.3		
79			25.9
80			38.5
81			58.1
82			27.8
83	84.9		
84	57.8		
85			35.2
86			1.1
87			1.5
88			4.0
89			2.1
90			7.8
91			7.6
92			5.0
93			1.4
94			55.2
95			14.1
96	55.0		
97	13.0		
98	13.0		
99			42.0
100			18.5
101			67.0
102	40.0		
103	18.0		
104			3.8
105	33.0		
106	20.0		
107			8.0
108			25.0
109			7.5
110			4.2

Compound	ExoU IC50(μ M)	SipB IC50(μ M)	SopE IC50(μ M)
111			23.0
112			16.6
113			14.5
114			7.3
115			61.6
116			15.5
117			15.5
118			100
119			47.4
120			22.7
121	25.0		
122	25.0		
123			30.0
124			16.0
125			20.0
126			3.0
127			10.0
128			100
129			16.0
130			31.0
131			1.5
132			42.0
133	100		
134			16.8
135			50.0
136			25.0
137	29.0		
138			100
139			24.0
140	48.0		
141			25.0
142	24.0		
143	40.0		
144			62.0
145	30.0		
146			35.0
147			60.0
148	33.0		
149	70.0		
150	30.0		
151			50.0
152			100.0
153			70.0
154	26.4		
155			28.0
156		50.0	
157	90.0		

Compound	ExoU IC50(μ M)	SipB IC50(μ M)	SopE IC50(μ M)
158	54.0		
159			100
160			16.0
161			56.0
162			57.0

The above compounds are listed only to provide examples that may be used in the methods of the invention. Based upon the instant disclosure, the skilled artisan would recognize other compounds intended to be included within the scope of the presently claimed invention that would be useful in the methods recited herein.

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

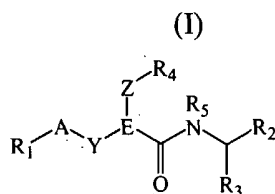
Although certain embodiments have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.

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10. Compounds That Inhibit the Binding of Integrins to Their Receptors, Scott,I.; Bore, R.; Biediger, R. J.; Grabbe, V.; Kassir, J.; Keller, K. M.; Lin. S.; Market, R.; Kogan, T. P.; WO99/52493, issued October 21, 1999.

WHAT IS CLAIMED:

1. A compound having the formula I:



wherein A is -C(O)- or -CH₂-;

Y is -NH- or -CH₂-;

E-Z is -C=CH- or -N-CH₂-;

R₁ is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, or substituted heteroaryl;

R₂ is hydrogen, carboxy, carboxymethyl, or hydroxymethyl;

R₃ is hydrogen, heterocyclyl, substituted heterocyclyl, heteroaryl, substituted heteroaryl, lower alkyl, substituted lower alkyl, aryl, or substituted aryl;

R₄ is aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, or substituted heterocyclyl;

R₅ is hydrogen or lower alkyl;

R₂ and R₃ can combine to form a C₄-C₈ cycloalkyl, optionally substituted by carboxy;

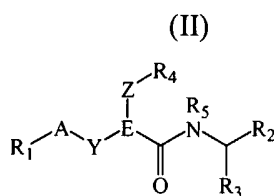
R₃ and R₅ can combine to form a heterocycle, optionally substituted at one to three positions thereof; with the following provisos:

if E is C, then A is -C(O)- and Y is CH₂; if E is N, then A is -C(O)- and Y is NH; and if E is N, A is -C(O)-, Y is NH and R₁ is aryl substituted with one to three moieties selected from the group consisting of C₁₋₄ alkyl, C₁₋₄ alkoxy, C₁₋₄ alkylthio, trifluoroalkyl, OH, Cl, Br, F, or carboxy, then R₃ is either hydrogen or a branched or straight chain C₁₋₆ alkyl group substituted with -OCOR₅, -OR₅, -SR₅, -SOR₅, -SO₂R₅, -NR₅R₆, -OCONR₅R₆, -NHCOR₅, -NHCOOR₅, -NHC(NH)NHNO₂, or -NHCONR₅R₆, wherein R₅ and R₆ are independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclo, aralkyl, heteroaralkyl, and heterocycloalkyl, or alternatively R₅

and R₆ may join to form a heterocyclic ring containing the nitrogen atom to which they are attached; or

an optical isomer, diastereomer or enantiomer thereof; or a pharmaceutically acceptable salt, hydrate, or prodrug thereof.

2. A method of treating a patient with an infection due to Gram-negative pathogenic bacteria by administering Type III protein secretion inhibitors having the formula (II):



wherein A is -C(O)- or -CH₂-;

Y is -NH- or -CH₂-;

E-Z is -C=CH- or -N-CH₂-;

R₁ is aryl, substituted aryl, heterocyclyl, substituted heterocyclyl, heteroaryl, or substituted heteroaryl;

R₂ is hydrogen, carboxy, carboxymethyl, or hydroxymethyl;

R₃ is hydrogen, heterocyclyl, substituted heterocyclyl, heteroaryl, substituted heteroaryl, lower alkyl, substituted lower alkyl, aryl, or substituted aryl;

R₄ is aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclyl, or substituted heterocyclyl;

R₅ is hydrogen or lower alkyl;

R₂ and R₃ can combine to form a C₄-C₈ cycloalkyl, optionally substituted by carboxy;

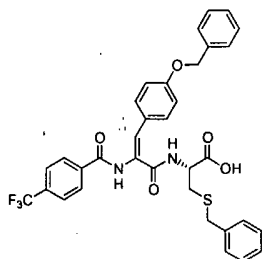
R₃ and R₅ can combine to form a heterocycle, optionally substituted at one to three positions thereof, with the following provisos:

if E is N, then A is -C(O)- and Y is NH, or A and Y are both CH₂; and if E is C, then A is -C(O)-; or

an optical isomer, diastereomer or enantiomer thereof; or a pharmaceutically acceptable salt, hydrate, or prodrug thereof.

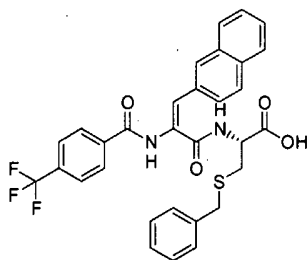
3. The compound of Claim 1 wherein X is N, Y is O, Z is C, and R₁ is bonded to Z.
4. The compound of Claim 1 wherein R₁ is selected from the group consisting of two ring fused heterocyclyl.
5. The compound of Claim 3 wherein R₁ is benzothienyl, benzofuryl, indolyl, quinolinyl, or quinoxalinyl.
6. The compound of Claim 1 wherein R₁ is biphenyl, or mono- or disubstituted phenyl.
7. The compound of Claim 1 wherein R₁ is substituted pyridinyl, or substituted piperidinyl.
8. The compound of Claim 1 wherein R₃ is benzyloxy, benzylthio, chlorobenzyl, benzylsulfoxo, or benzylsulfinyl.
9. The compound of Claim 1 wherein R₄ is substituted phenyl or naphthyl.
10. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of a compound according to Claim 1 to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

11. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



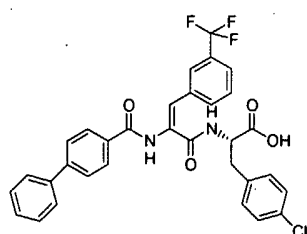
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

12. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



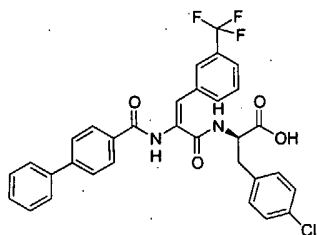
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

13. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



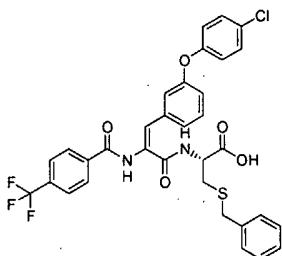
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

14. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



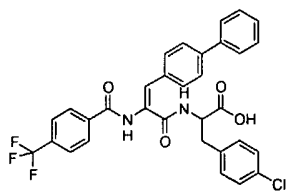
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

15. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



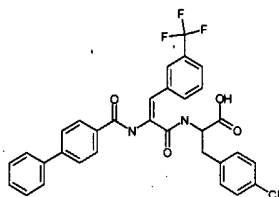
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

16. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



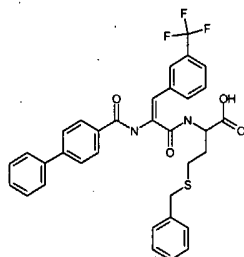
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

17. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



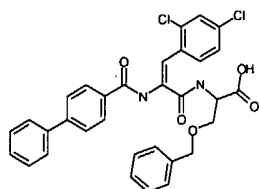
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

18. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



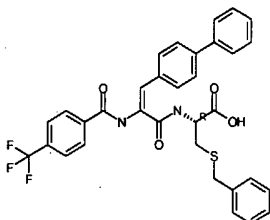
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

19. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



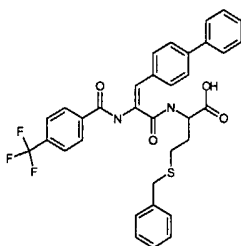
to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

20. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

21. A method of inhibiting bacteria with Type III protein secretion systems, said method comprising administration of an effective amount of



to a subject in need of treatment for infection by said bacteria with Type III protein secretion systems.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/015807

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K5/065 C07C235/78 C07C323/59 C07C281/06 C07C237/22
C07C323/25 C07K5/078 C07C275/10 A61K38/05 A61K31/166
A61P31/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEILSTEIN Data, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/022775 A (INNATE PHARMACEUTICALS) 18 March 2004 (2004-03-18) page 9; claims 16-21, 24-29; figures 8-12	1-21
X	----- DATABASE CAPLUS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; ISHII, TADAO ET AL: "Nitrofurans" XP002385358 retrieved from STN Database accession no. 1970:111284 abstract & JP 45 002741 B (MEIJI CONFECTIONARY) 29 January 1970 (1970-01-29) ----- -/--	1, 2, 6, 10

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

15 June 2006

Date of mailing of the international search report

04/07/2006

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Authorized officer

English, R

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2005/015807

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A.I. HASHEM, ET AL.: "Preparation and reactions of some derivatives of acrylic acid hydrazides and amides" JOURNAL FUER PRAKTISCHE CHEMIE, vol. 323, no. 1, 1981, pages 164-168, XP002385368 WILEY VCH, WEINHEIM, DE compounds 5A-D	1,6
X	----- R. FILLER, ET AL.: "Chemistry of lactones. III. Reactions of alpha-benzylidene-gamma-phenyl-delta-beta,gamma-butenolide" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 81, no. 2, 20 January 1959 (1959-01-20), pages 391-393, XP002385369 AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US compounds V,VI	1
X	----- G.H. SAYED, ET AL.: "Some reactions of alpha-3-benzylidene-5-(p-bromophenyl)furan-(2H)-2-one in comparison with 4-benzylidene-1-aryl-2-(p-bromophenyl)-2-pyrrolinen-5-ones" EGYPTIAN JOURNAL OF CHEMISTRY, vol. 22, no. 4, 1979, pages 285-295, XP008065291 CAIRO, EG compound IID	1,6
X	----- M.A. AL-HAIZA, ET AL.: "Reactions of 3-(p-phenylbenzoyl)propionic acid with aromatic aldehydes and some nitrogen nucleophiles" EGYPTIAN JOURNAL OF CHEMISTRY, vol. 42, no. 1, 1999, pages 83-90, XP008065290 CAIRO, EG compounds (IV)A-H ----- -/--	1,6,9

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/015807

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M.S.Y. KHAN, ET AL.: "Studies on butenolides: 2-arylidene-4-(substituted aryl)but-3-en-4-olides. Synthesis, reactions and biological activity" INDIAN JOURNAL OF CHEMISTRY, SECTION B: ORGANIC, INCL. MEDICINAL, vol. 41B, no. 10, October 2002 (2002-10), pages 2160-2171, XP009038639 PUBLICATIONS & INFORMATIONS DIRECTORATE, NEW DELHI, IN ISSN: 0019-5103 Intermediates between butenolides 11,10,21,27,28 and N-benzylpyrrolones 43-47	1,6,9
X	M.S.Y. KHAN, ET AL.: "Synthesis and reactions of some new 2-arylidene-4-(biphenyl-4-yl)but-3-en-4-olides with a study of their biological activity" DIE PHARMAZIE, vol. 57, no. 7, July 2002 (2002-07), pages 448-452, XP002385393 GOVI VERLAG, ESCHBORN, DE Intermediates between butenolides 9,14,4,5,16 and N-benzylpyrrolones 28-32	1,9
X	G.V. BOYD, ET AL.: "Synthesis and reactions of 2-dialkylaminofurans" JOURNAL OF THE CHEMICAL SOCIETY, PERKIN TRANSACTIONS 1, no. 21, 1973, pages 2523-2531, XP002385395 ROYAL SOCIETY OF CHEMISTRY, LETCHWORTH, GB compound 14A	1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/015807

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 2,10-21 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2005/015807

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004022775 A	18-03-2004	AU 2003256215 A1 EP 1543148 A1	29-03-2004 22-06-2005
JP 45002741 B		NONE	