CASPASE INHIBITORS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Application 60/392,592 filed June 28, 2002 and United States Provisional Application 60/435,073, filed December 20, 2002, the entirety of both documents being incorporated herein by reference.

FIELD OF THE INVENTION

[0002] This invention is in the field of medicinal chemistry and relates to compounds, and pharmaceutical compositions thereof, that inhibit caspases that mediate cell apoptosis and inflammation. The invention also relates to processes for preparing the compounds and to methods of using the compounds and pharmaceutical compositions of this invention to treat diseases where caspase activity is implicated.

BACKGROUND OF THE INVENTION

[0003] Apoptosis, or programmed cell death, is a principal mechanism by which organisms eliminate unwanted cells. The deregulation of apoptosis, either excessive apoptosis or the failure to undergo it, has been implicated in a number of diseases such as cancer, acute inflammatory and autoimmune disorders, ischemic diseases and certain neurodegenerative disorders (see generally *Science*, **281**, 1283-1312 (1998); Ellis et al., *Ann. Rev. Cell. Biol.*, **7**, 663 (1991)).

[0004] Caspases are a family of cysteine protease enzymes that are key mediators in the signaling pathways for apoptosis and cell disassembly (Thornberry, *Chem.*

Biol., **5**, R97-R103 (1998)). These signaling pathways vary depending on cell type and stimulus, but all apoptosis pathways appear to converge at a common effector pathway leading to proteolysis of key proteins. Caspases are involved in both the effector phase of the signaling pathway and further upstream at its initiation. The upstream caspases involved in initiation events become activated and in turn activate other caspases that are involved in the later phases of apoptosis.

[0005] Caspase-1, the first identified caspase, is also known as interleukin converting enzyme or "ICE." Caspase-1 converts precursor interleukin-1β ("pIL-1β") to the pro-inflammatory active form by specific cleavage of pIL-1β between Asp-116 and Ala-117. Besides caspase-1 there are also eleven other known human caspases, all of which cleave specifically at aspartyl residues. They are also observed to have stringent requirements for at least four amino acid residues on the N-terminal side of the cleavage site.

[0006] The caspases have been classified into three groups depending on the amino acid sequence that is preferred or primarily recognized. The group of caspases, which includes caspases 1, 4, 5 and 13, have been shown to prefer hydrophobic aromatic amino acids at position 4 on the N-terminal side of the cleavage site. Another group that includes caspases 2, 3 and 7, recognize aspartyl residues at both positions 1 and 4 on the N-terminal side of the cleavage site, and preferably a sequence of Asp-Glu-X-Asp. A third group, which includes caspases 6, 8, 9 and 10, tolerate many amino acids in the primary recognition sequence, but seem to

prefer residues with branched, aliphatic side chains such as valine and leucine at position 4.

[0007] The caspases have also been grouped according to their perceived function. The first subfamily consists of caspases-1 (ICE), 4, 5 and 13. These caspases have been shown to be involved in pro-inflammatory cytokine processing and therefore play an important role in inflammation. Caspase-1, the most studied enzyme of this class, activates the IL-1β precursor by proteolytic cleavage. This enzyme therefore plays a key role in the inflammatory response. Caspase-1 is also involved in the processing of interferon-γ inducing factor (IGIF) which stimulates the production of interferon gamma, a key immunoregulator that modulates antigen presentation, T-cell activation and cell adhesion.

[0008] The remaining caspases make up the second and third subfamilies. These enzymes are of central importance in the intracellular signaling pathways leading to apoptosis. One subfamily consists of the enzymes involved in initiating events in the apoptotic pathway, including transduction of signals from the plasma membrane. Members of this subfamily include caspases-2, 8, 9 and 10. The other subfamily, consisting of the effector caspases 3, 6 and 7, are involved in the final downstream cleavage events that result in the systematic breakdown and death of the cell by apoptosis. Caspases involved in the upstream signal transduction activate the downstream caspases, which then disable DNA repair mechanisms, fragment DNA, dismantle the cell cytoskeleton and finally fragment the cell.

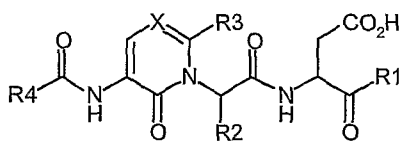
[0009] Knowledge of the four amino acid sequence primarily recognized by the caspases has been used to design caspase inhibitors. Reversible tetrapeptide inhibitors have been prepared having the structure CH_3CO-[P4]-[P3]-[P2]-CH(R)CH_2CO_2H where P2 to P4 represent an optimal amino acid recognition sequence and R is an aldehyde, nitrile or ketone capable of binding to the caspase cysteine sulfhydryl. Rano and Thornberry, *Chem. Biol.* **4**, 149-155 (1997); Mjalli, et al., *Bioorg. Med. Chem. Lett.* **3**, 2689-2692 (1993); Nicholson et al., *Nature* **376**, 37-43 (1995). Irreversible inhibitors based on the analogous tetrapeptide recognition sequence have been prepared where R is an acyloxymethylketone $-COCH_2OCOR'$. R' is exemplified by an optionally substituted phenyl such as 2,6-dichlorobenzoyloxy and where R is $COCH_2X$ where X is a leaving group such as F or Cl. Thornberry et al., *Biochemistry* **33**, 3934 (1994); Dolle et al., *J Med. Chem.* **37**, 563-564 (1994).

[0010] The utility of caspase inhibitors to treat a variety of mammalian disease states associated with an increase in cellular apoptosis has been demonstrated using peptidic caspase inhibitors. For example, in rodent models caspase inhibitors have been shown to reduce infarct size and inhibit cardiomyocyte apoptosis after myocardial infarction, to reduce lesion volume and neurological deficit resulting from stroke, to reduce post-traumatic apoptosis and neurological deficit in traumatic brain injury, to be effective in treating fulminant liver destruction, and to improved survival after endotoxic shock. Yaoita et al., *Circulation*, **97**, 276 (1998); Endres et al., *J Cerebral Blood Flow and Metabolism*, **18**, 238, (1998); Cheng et al., J. Clin. Invest., **101**, 1992 (1998); Yakovlev et al., *J*

Neuroscience, **17**, 7415 (1997); Rodriquez et al., *J. Exp. Med.*, **184**, 2067 (1996); Grobmyer et al., *Mol. Med.*, **5**, 585 (1999).

[0011] In general, the peptidic inhibitors described above are very potent against some of the caspase enzymes. However, this potency has not always been reflected in cellular models of apoptosis. In addition peptide inhibitors are typically characterized by undesirable pharmacological properties such as poor oral absorption, poor stability and rapid metabolism. Plattner and Norbeck, in *Drug Discovery Technologies*, Clark and Moos, Eds. (Ellis Horwood, Chichester, England, 1990).

[0012] Recognizing the need to improve the pharmacological properties of the peptidic caspase inhibitors, peptidomimetic inhibitors have been reported. Amongst these, inhibitors where the P3 amino acid has been replaced by derivatives of 3-aminopyridin-2-ones and 5-aminopyrimidin-4-ones have received much attention (US Patent 5,756,466 (Bemis et al.); Dolle et al. *J. Med. Chem.* **39**, 2438, (1996); Golec et al. *Bioorg. Med. Chem. Lett.* **7**, 2181, (1997); Semple et al, *Biorg. Med. Chem. Lett.* **7**, 1337, (1997) involving compounds of general structure:



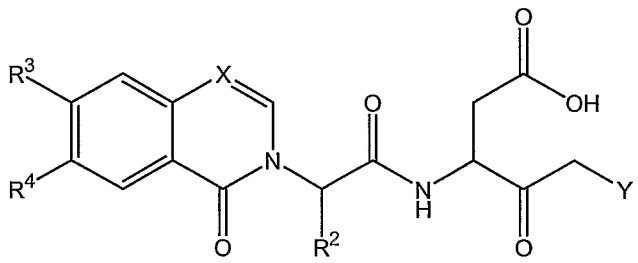
wherein R_1-R_4 and X are various groups.

[0013] Due to the inherent problems of the peptidic inhibitors, there continues to be a need for small molecule, nonpeptide caspase inhibitors that are potent, stable, and penetrate membranes to provide effective

inhibition of apoptosis *in vivo*. Such compounds would be extremely useful in treating the aforementioned diseases where caspase enzymes play a role. WO 01/42216 discloses caspase inhibitors and uses thereof. The present invention provides a selection over the WO 01/42216 disclosure.

Summary of the Invention

[0014] The present invention provides a compound of formula **I**:



I

wherein:

X is CH or N;

Y is halo, trifluorophenoxy, or tetrafluorophenoxy;

R^2 is a C_{1-6} straight chained or branched alkyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3; and

R^4 is hydrogen, halo, OCF_3, SR, CN, CF_3, Ar, or T-Ar;

wherein:

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

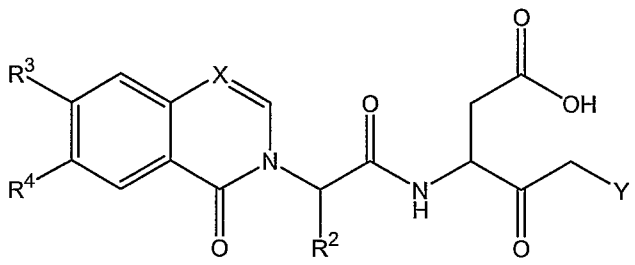
R^5 and R^6 each is independently H or C_{1-6} alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6} alkyl);

provided that when Y is halo, then both, R^3 and R^4, are not simultaneously hydrogen.

[0015] The present invention also provides pharmaceutical compositions and methods using such compositions for treating a caspase-mediated disease.

Detailed Description of the Invention

[0016] The present invention provides a compound of formula **I**:



I

wherein:

X is CH or N;

Y is halo, trifluorophenoxy, or tetrafluorophenoxy;

R^2 is a C_{1-6} straight chained or branched alkyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3; and

R^4 is hydrogen, halo, OCF_3, SR, CN, CF_3, Ar, or T-Ar;

wherein:

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl);

provided that when Y is halo, then both, R^3 and R^4, are not simultaneously hydrogen.

[0017] In another embodiment of formula I, R^4 is hydrogen, halo, OCF_3, CN, CF_3, or T-Ar; wherein T, Ar, and the other variables are as defined above.

[0018] The compounds of the present invention represent a selection over the genus of International PCT Publication WO 01/42216, the entire disclosure of which is incorporated herein. Specifically, the compounds of the present invention have an unexpected and surprising ability to inhibit apoptosis and/or inhibit Il-1ß release from activated cells.

[0019] According to a preferred embodiment, R^2 in formula I is ethyl, n-propyl, or isopropyl.

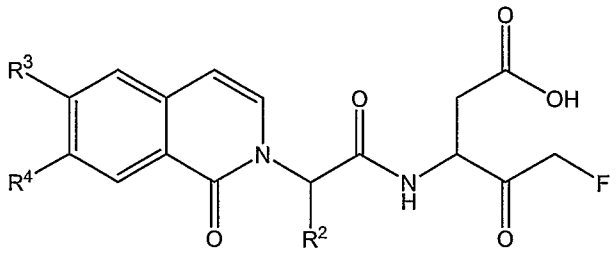
[0020] According to a more preferred embodiment, R^2 in formula I is ethyl.

[0021] According to an even more preferred embodiment, R^2 in formula I is (S)-ethyl.

[0022] According to a preferred embodiment, R^3 in formula I is hydrogen.

0023] According to a preferred embodiment, Y in formula I is F, trifluorophenoxy, or tetrafluorophenoxy.

[0024] According to another preferred embodiment, the present invention provides a compound of formula IA:



IA

wherein:

R^2 is ethyl, n-propyl, or isopropyl; and

R^3 and R^4 are each independently hydrogen, halo, OCF_3, CN, CF_3, or Ar, provided that both R^3 and R^4 are not simultaneously hydrogen;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl);

[0025] According to another embodiment of formula IA, R^3 and R^4 are each independently hydrogen, halo, OCF_3, CN, or CF_3, provided that both, R^3 and R^4, are not simultaneously hydrogen; and R^2 is as defined above for formula IA.

[0026] According to a preferred embodiment, R^2 in the compound of formula IA is ethyl.

[0027] According to a more preferred embodiment, R^2 in formula IA is (S)-ethyl.

[0028] According to another preferred embodiment, R^2 in the compound of formula IA is isopropyl.

[0029] According to a preferred embodiment, R^3 in the compound of formula IA is hydrogen.

[0030] According to a preferred embodiment, R^4 in the compound of formula IA is F, Cl, CN, Ar, or CF_3.

[0031] According to a more preferred embodiment, R^4 in the compound of formula IA is Cl.

[0032] According to another more preferred embodiment, R^4 in the compound of formula IA is CF_3.

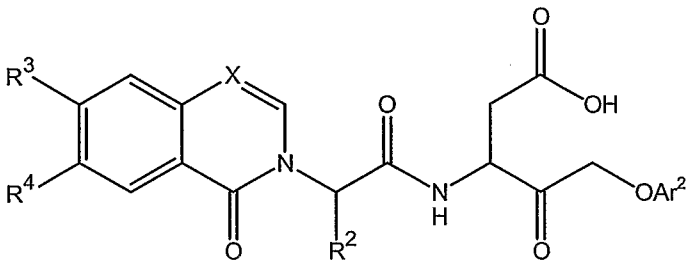
[0033] According to another preferred embodiment, R^3 in the compound of formula IA is hydrogen, and R^4 is F, Cl, CN, Ar, or CF_3.

[0034] According to another preferred embodiment, R^3 in the compound of formula IA is hydrogen and R^4 is Cl.

[0035] According to another preferred embodiment, R^3 in the compound of formula IA is hydrogen and R^4 is CF_3.

[0036] According to another preferred embodiment, R^3 in the compound of formula IA is hydrogen and R^4 is Ar.

[0037] According to another preferred embodiment, the present invention provides a compound of formula IB:



IB

wherein:

X is CH or N;

R^2 is ethyl, n-propyl, or isopropyl;

R^3 and R^4 are each independently hydrogen, halo, OCF_3, CN, or CF_3; and

Ar^2 is trifluorophenyl or tetrafluorophenyl.

[0038] According to a preferred embodiment, Ar^2 in the compound of formula IB is 2,3,5,6-tetrafluorophenyl.

[0039] According to a preferred embodiment, R^2 in the compound of formula IB is ethyl.

[0040] According to a more preferred embodiment, R^2 in formula IB is (S)-ethyl.

[0041] According to a preferred embodiment, X in the compound of formula IB is CH.

[0042] According to a preferred embodiment, R^3 in the compound of formula IB is hydrogen.

[0043] According to a preferred embodiment, R^4 in the compound of formula IB is F, Cl, or CF_3.

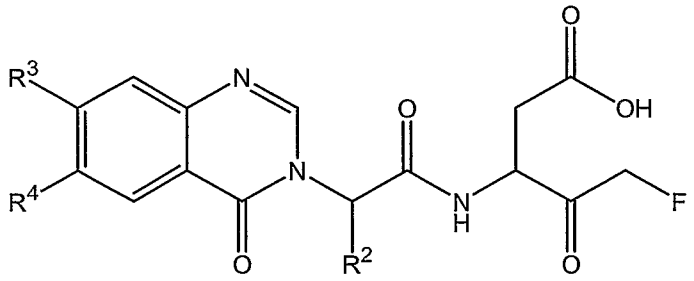
[0044] According to a more preferred embodiment, R^4 in the compound of formula IB is Cl.

[0045] According to a more preferred embodiment, R^4 in the compound of formula IB is CF_3.

[0046] According to a preferred embodiment, R^3 in the compound of formula IB is hydrogen, and R^4 is F, Cl, or CF_3.

[0047] According to another preferred embodiment, Ar^2 in the compound of formula IB is 2,3,5,6-tetrafluorophenyl, R^3 is hydrogen, and R^4 is Cl.

[0048] According to another preferred embodiment, the present invention provides a compound of formula IC:



IC

wherein:

R^2 is ethyl, n-propyl, or isopropyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3;

R^4 is halo, OCF_3 CN, CF_3, SR, or T-Ar;

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally

containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl).

[0049] According to a preferred embodiment of the compound of formula IC, R^4 is T-Ar;

[0050] According to a preferred embodiment, R^2 in the compound of formula IC is ethyl.

[0051] According to a more preferred embodiment, R^2 in formula IC is (S)-ethyl.

[0052] According to a preferred embodiment, R^3 in the compound of formula IC is hydrogen.

[0053] According to a preferred embodiment, R^4 in the compound of formula IC is F, Cl, CN, or CF_3.

[0054] According to a more preferred embodiment, R^4 in the compound of formula IC is Cl.

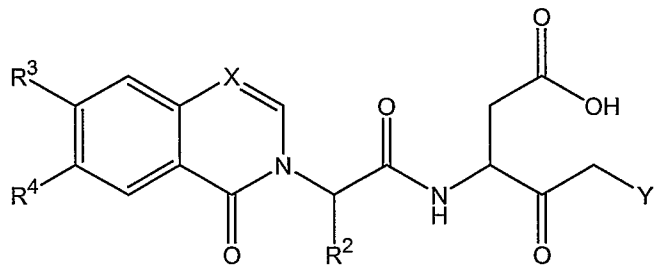
[0055] According to a preferred embodiment, T in the compound of formula IC is O.

[0056] According to a preferred embodiment, Ar if present in the compound of formula IC, is optionally substituted with CF_3 or halo (preferably, the halo is chloro or fluoro).

[0057] As used herein, "C_{1-6}" indicates the presence of 1, 2, 3, 4, 5, or 6 carbon atoms (or optional heteroatom substituted therefor).

[0058] According to a more preferred embodiment, the present invention provides a compound of formula I, selected from Table 1 below:

Table 1



(I)

Example	R^4	R^3	R^2	Y	X
1	Cl	H	Et	F	CH
2	CF_3	H	Et	F	CH
3	H	Cl	Et	F	CH
4	H	CF_3	Et	F	CH
5	Cl	H	Et	2,3,5,6-tetrafluoro phenoxy	N
6	H	H	Et	2,3,5,6-tetrafluoro phenoxy	N
7	H	H	Et	2,4,6-trifluoroph enoxy	N
8	H	H	Et	2,3,6-trifluoroph enoxy	N
9	H	H	Et	2,3,5,6-tetrafluoro phenoxy	CH
10	Cl	H	Et	2,3,5,6-tetrafluoro phenoxy	CH

Example	R^4	R^3	R^2	Y	X
11	Cl	Cl	Et	2,3,5,6-tetrafluoro phenoxy	CH
12	2-chloro-phenoxy	H	Et	F	N
13	3-chloro-phenoxy	H	Et	F	N
14	3-fluoro-phenoxy	H	Et	F	N
15	2,4-dichloro-phenoxy	H	Et	F	N
16	phenylsulfanyl	H	Et	F	N
17	3-fluoro-phenylsulfanyl	H	Et	F	N
18	Cl	H	i-Pr	F	CH
19	CF_3	H	Et	2,3,5,6-tetrafluoro phenoxy	CH
20	CF_3	H	Et	2,3,5,6-tetrafluoro phenoxy	N
21	phenyl	H	Et	F	CH
22	S(n-Pr)	H	Et	F	N

[0059] According to another embodiment, the present invention provides a pharmaceutical composition comprising:

a) a compound of formula I, as defined above, or a pharmaceutically acceptable salt thereof; and

b) a pharmaceutically acceptable carrier, adjuvant or vehicle.

[0060] According to a preferred embodiment, the pharmaceutical composition of the present invention comprises:

a) a compound of formula IA, formula IB, or formula IC; and

b) a pharmaceutically acceptable carrier, adjuvant or vehicle.

[0061] According to a more preferred embodiment, the pharmaceutical composition of the present invention comprises a compound selected from Table 1 above.

[0062] Compounds of this invention may exist in solution as either the open form 1 or the cyclized hemiketal form 2. The representation herein of either isomeric form is meant to include the other.



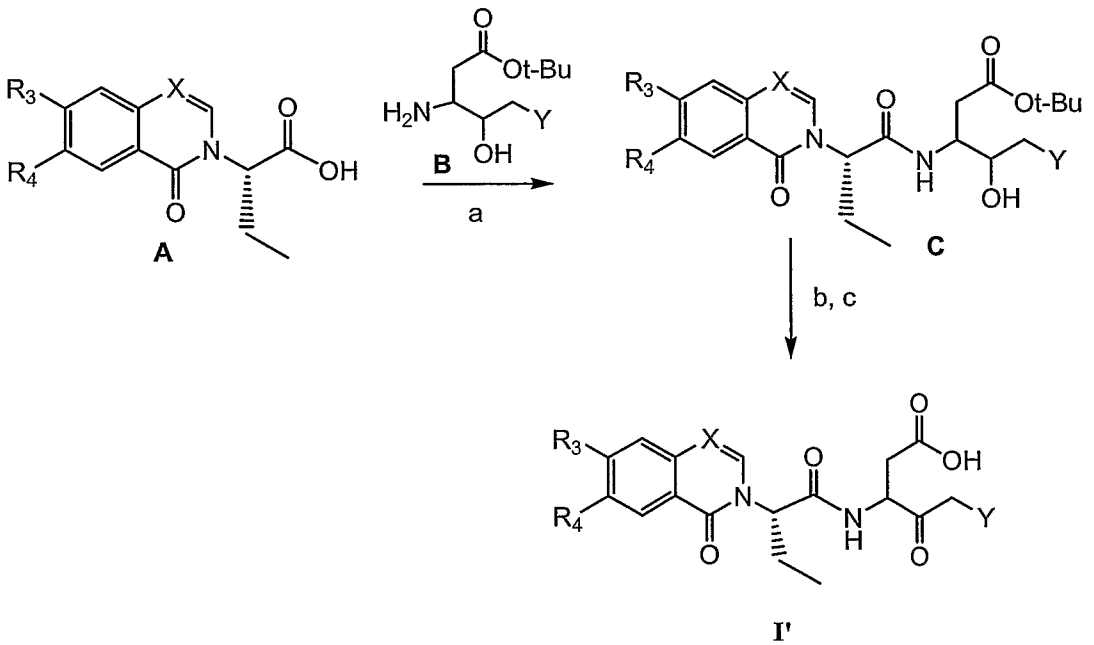
Form 1 Form 2

[0063] It will be apparent to one skilled in the art that certain compounds of this invention may exist in tautomeric forms or hydrated forms, all such forms of the compounds being within the scope of the invention. Unless otherwise stated, structures depicted herein are also meant to include all stereochemical forms of the structure; i.e., the R and S configurations for each asymmetric center. Therefore, single stereochemical isomers as well as enantiomeric and diastereomeric mixtures of the present compounds are within the scope of the invention. Unless otherwise stated, structures

depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are within the scope of this invention.

[0064] The compounds of this invention may be prepared in general by methods known to those skilled in the art for analogous compounds. Synthetic routes to the compounds of the present invention are generically described in WO 01/42216. For the purposes of illustration, the following Schemes I-IV for the synthesis of the compounds of the present invention are provided.

Scheme I

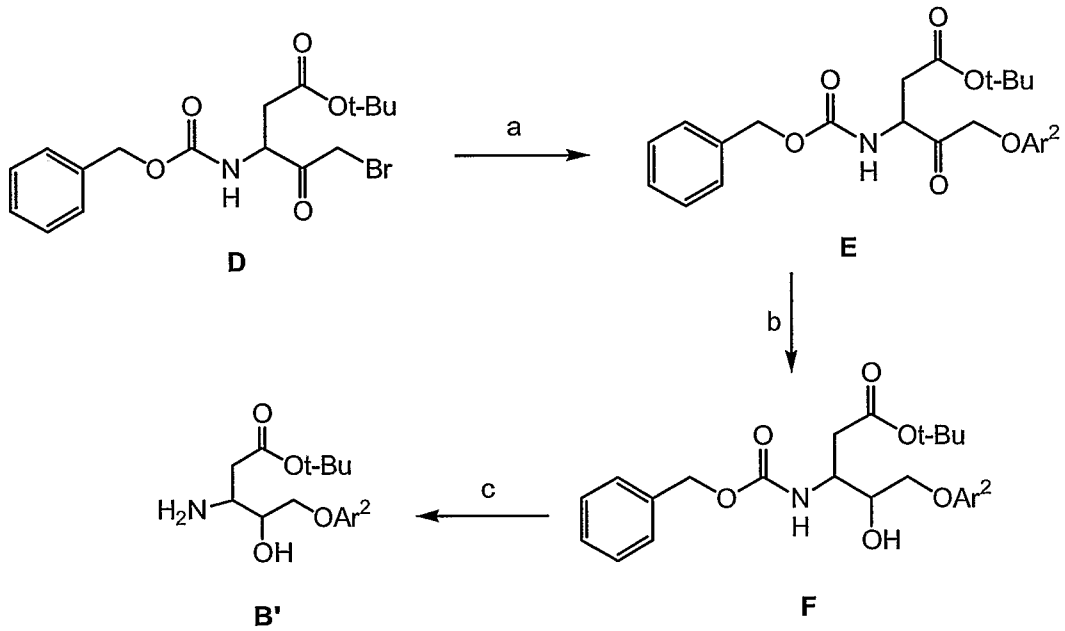


Reagents: (a) EDC/DMAP/HOBt/THF; (b) Dess-Martin periodinane; (c) TFA/DCM

[0065] In Scheme I above, the following abbreviations are used: EDC is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HOBt is 1-hydroxybenzotriazole; THF is tetrahydrofuran; TFA is trifluoroacetic acid; DCM is dichloromethane; DMAP is 4-dimethylaminopyridine. Acid **A** is coupled to amino alcohol **B** to give amide **C** . The hydroxy group in compound **C** is oxidized to give the ketone followed by acid hydrolysis of the tert butyl ester to give **I'** as the free acid.

[0066] In the case of fluoromethyl ketones where CH_2Y is CH_2F, the amino alcohol **B** may be obtained according to the method of Revesz et al., *Tetrahedron Lett.* **35**, 9693 (1994). In the case of fluoro-substituted phenoxy ketones where Y in CH_2Y is 2,3,5,6-tetrafluorophenoxy, 2,4,6-trifluorophenoxy, or 2,3,6-trifluorophenoxy, the amino alcohol **B** may be obtained by methods analogous to those of Semple et al., *Bioorganic and Medicinal Chemistry Letters*, **7**, 1337 (1997) (Scheme II).

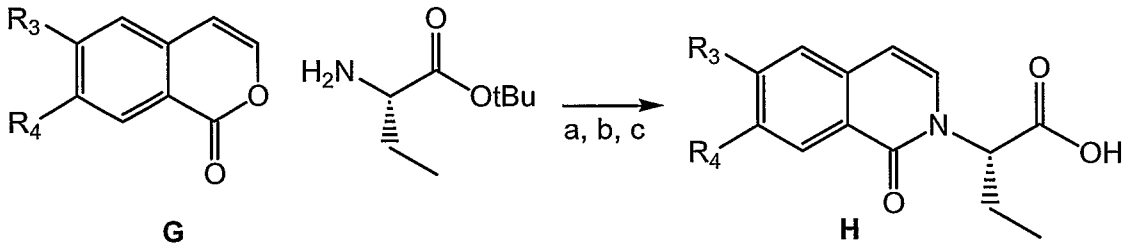
Scheme II



Reagents: (a)KF/DMF/Ar^2OH; (b) $NaBH_4$/THF; (c) H_2/Pd/C/MeOH

[0067] In scheme II above, the following abbreviations are used: KF is potassium fluoride; DMF is N,N-dimethylformamide; Ar^2OH is either 2,3,5,6-tetrafluorophenol, 2,4,6-trifluorophenol or 2,3,6-trifluorophenol; THF is tetahydrofuran; MeOH is methanol. Commercially available bromoketone **D** is reacted with the appropriately substituted fluorophenol and potassium fluoride to give phenoxy ketone **E**. The ketone is then reduced with sodium borohydride to give the alcohol **F**, which is hydrogenated using palladium on carbon as catalyst to give the amino alcohol **B'** (Ar^2=fluoro-substituted phenyl).

Scheme III

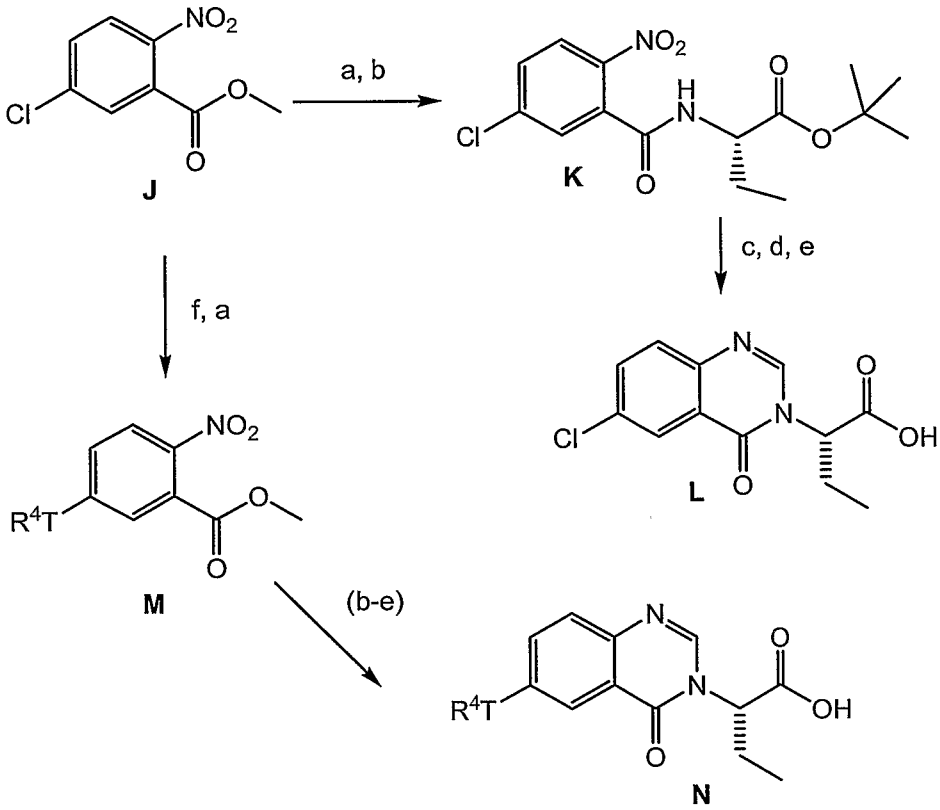


G H

Reagents: (a) heat; (b) conc. HCl/IPA; (c) TFA/DCM

[0068] In Scheme III above, the following abbreviations are used: IPA is isopropyl alcohol; TFA is trifluoroacetic acid and DCM is dichloromethane. Isoquinolin-1-one acid derivatives (in formula **I**, X=C) can be prepared in chiral form using the synthetic sequence shown in Scheme III. The starting isocoumarin **G** is prepared by methods analogous to Narasimhan et al., *Synthesis*, 797 (1975) and Margaretha et al., *Tetrahedron* **56**, 6763 (2000) unless stated otherwise. Isocoumarin **G** is first heated with commercially available (S)-2-aminobutyric acid, *tert*-butyl ester. The resulting compound is reacted with concentrated hydrochloric acid in isopropanol to give the isoquinolin-1-one *tert*-butyl ester which is deprotected to provide the acid **H** using trifluoroacetic acid. The acid is then coupled to amino alcohol **B** (Scheme I).

Scheme IV



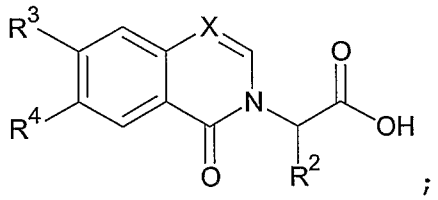
Reagents: (a) LiOH/THF/H_2O; (b) (S)-2-Aminobutyric acid, *tert*-butyl ester/EDC/HOBt/DMAP/THF; (c) $SnCl_2$/MeOH; (d) Trimethylorthoformate/AcOH/reflux; (e) TFA/DCM; (f) R^4-TH/K_2CO_3/DMF.

[0069] In scheme IV above, the following abbreviations are used: AcOH is acetic acid and R^4-TH can be 2-chlorophenol, 3-chlorophenol, 3-fluorophenol or 2,4-dichlorophenol or thiophenol or 3-fluorothiophenol. (4-oxo-4*H*-quinazolin-3-yl) acid derivatives **A** (X=N) are prepared in chiral form using methods analogous to Makino et al., *Synlett*, **11**, 1670 (2000). Commercially avaliable 5-chloro-2-nitrobenzoic acid methyl ester **J** is hydrolysed to the acid using lithium hydroxide and the resulting

acid coupled to commercially avaliable (S)-2-aminobutyric acid *tert*-butyl ester to give amide **K**. Reduction of the nitro group using tin (II) chloride followed by acid catalysed cyclo-condensation with trimethylorthoformate furnishes the quinazoline, which is deprotected with trifluoroacetic acid to give the 4-oxo-4*H*-quinazolin-3-yl acid **L.** The acid is then coupled to the amino alcohol **B** (Scheme I). Alternatively, 5-chloro-2-nitrobenzoic acid methyl ester **J** can be reacted with a suitably substituted phenol or thiophenol by heating with potassium carbonate in DMF to give the corresponding 6-phenoxy (Y=O) or 6-phenylsulfanyl (Y=S) derivative. Treatment with lithium hydroxide gives the acid **M**, which can be further elaborated (Scheme IV, steps b-e) to give 4-oxo-4*H*-quinazolin-3-yl acid **N** which is then coupled to amino alcohol **B** as previously described. 2-Nitrobenzoic acid may also be elaborated in an analogous manner to furnish the unsubstituted 4-oxo-4*H*-quinazolin-3-yl acid.

[0070] Accordingly one aspect of this invention relates to a general method of preparing a compound of formula I, comprising the steps of:

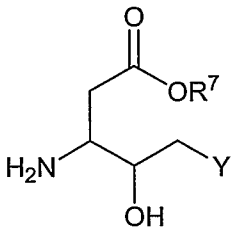
reacting an acid or acid derivative of formula II,



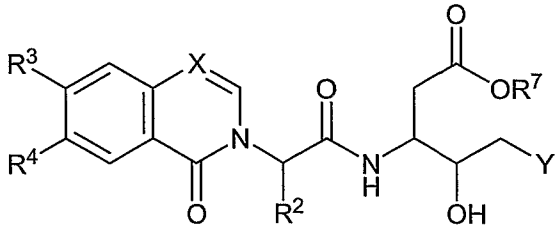
;

II

with an amino alcohol of formula B to provide a compound of formula III,



B



III; and

converting intermediate III to compound I, wherein; R^7 is a suitable protecting group, and Y, X, R^2, R^3, and R^4 are as described in any of the embodiments herein. A suitable protecting group would be known to skilled practitioners (see e.g., "Protective Groups in Organic Synthesis" Third Ed. Greene, T. W. and Wuts, P. G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which is hereby incorporated by reference). Preferably R^7 is a C_{1-6} straight or branched alkyl. More preferably, R^7 is t-butyl.

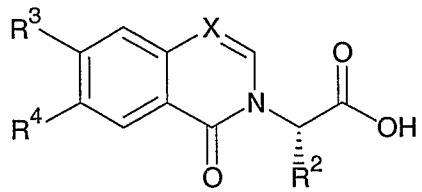
[0071] The compound of formula II and the compound of formula B may be reacted under any conditions for coupling an amino compound and an acid compound. Such conditions are well known to skilled practitioners. Preferred coupling conditions are those described in the schemes and examples herein.

[0072] The compound of formula III may be converted to a compound of formula I under any conditions for converting a hydroxy group to a carbonyl group and converting a protected acid to the free acid. Such conditions are well known to skilled practitioners. Preferred conditions for preparing a carbonyl group from a hydroxyl group are those oxidizing conditions described in the schemes and examples herein. Preferred conditions for deprotecting a protected acid when R^7 is t-butyl are

those hydrolysis conditions described in the schemes and examples herein.

[0069] This method is particularly useful for preparing chiral compounds of this invention, where the carbon bearing the R^2 substituent is stereochemically enriched. As exemplified below (see e.g., Examples 1-22), intermediate acids or acid derivatives of formula II may be obtained in chiral form. This is illustrated herein for quinazolin-4-ones where X is nitrogen (see e.g., Examples 5-8, 12-18, 20, and 22) and for isoquinolin-1-ones where X is CH (see e.g., Examples 1-4, 9-11, 19, and 21). The coupling of II and B to provide III may be carried out according to any suitable method. The conversion of III to provide I may be performed as described herein or according to other methods familiar to those skilled in the art.

[0074] Certain chiral intermediates of II are useful in processes for preparing compounds of this invention. A preferred intermediate is represented by compound IIA:



(S)-IIA

wherein X, R^2, R^3, and R^4 are as described in any of the embodiments herein.

[0075] According to a more preferred embodiment for compounds of formula II and IIA, R^2 is ethyl or isopropyl.

[0076] The compounds of this invention can be assayed for their ability to inhibit apoptosis, the release of IL-1β or caspase activity directly. Assays for each of

the activities are known in the art and are described below in detail in Examples 23-25.

[0077] If pharmaceutically acceptable salts of the compounds of this invention are utilized in these compositions, those salts are preferably derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzene sulfonate, bisulfate, butyrate, citrate, camphorate, camphor sulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenyl-propionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts, such as sodium and potassium salts, alkaline earth metal salts, such as calcium and magnesium salts, salts with organic bases, such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth.

[0078] Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates, such as dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides, such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

[0079] The compounds utilized in the compositions and methods of this invention may also be modified by appending appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase solubility to allow administration by injection, alter metabolism and alter rate of excretion.

[0080] Pharmaceutically acceptable carriers that may be used in these compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[0081] According to a preferred embodiment, the compositions of this invention are formulated for pharmaceutical administration to a mammal, preferably a human being.

[0082] Such pharmaceutical compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term

"parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the compositions are administered orally or intravenously.

[0083] Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. 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 are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents, which are commonly, used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly

used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0084] The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers, which are commonly used, include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0085] Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient, which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

[0086] The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

[0087] Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

[0088] For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0089] For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with our without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

[0090] The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline,

employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

[0091] The above-described compositions are particularly useful in therapeutic applications relating to an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, retinal disorders, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, organ apoptosis after burn injury, osteoporosis, leukemia's and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary

artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease, renal disease, polycystic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, an immunotherapy for the treatment of various forms of cancer, organ failure, and meningitis. The compounds and compositions are also useful in treating complications associated with coronary artery bypass grafts. The amount of compound present in the above-described compositions should be sufficient to cause a detectable decrease in the severity of the disease or in caspase activity and/or cell apoptosis, as measured by any of the assays known in the art.

[0092] According to another embodiment, the compositions of this invention may further comprise another therapeutic agent. Such agents include, but are not limited to, thrombolytic agents such as tissue plasminogen activator and streptokinase. When a second agent is used, the second agent may be administered either as a separate dosage form or as part of a single dosage form with the compounds or compositions of this invention. Both the compound and the additional agent should be present at dosage levels of between 10 to 100%, and more preferably between about 10 to 80% of the dosage normally administered in a monotherapy regimen.

[0093] It should also be understood that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the

activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of active ingredients will also depend upon the particular compound and other therapeutic agent, if present, in the composition.

[0094] In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels of about 0.01 mg/kg to about 50 mg/kg and preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect.

[0095] In a preferred embodiment, the invention provides a method of treating a mammal, having one of the aforementioned diseases, comprising the step of administering to said mammal a pharmaceutically acceptable composition described above. In this embodiment, if the patient is also administered another therapeutic agent or caspase inhibitor, it may be delivered together with the compound of this invention in a single dosage form, or, as a separate dosage form. When administered as a separate dosage form, the other caspase inhibitor or agent may be administered prior to, at the same time as, or following administration of a pharmaceutically acceptable composition comprising a compound of this invention.

[0096] The compounds of this invention or pharmaceutically acceptable compositions thereof may also be incorporated into compositions for coating implantable medical devices, such as prostheses, artificial valves, vascular grafts, stents and catheters. Accordingly, the

present invention, in another aspect, includes a composition for coating an implantable device comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device. In still another aspect, the present invention includes an implantable device coated with a composition comprising a compound of the present invention as described generally above, and in classes and subclasses herein, and a carrier suitable for coating said implantable device.

0097] Another aspect of the invention relates to inhibiting caspase activity in a biological sample or a patient, which method comprises administering to the patient, or contacting said biological sample with a compound of formula **I** or a composition comprising said compound. The term "biological sample", as used herein, includes, without limitation, cell cultures or extracts thereof; biopsied material obtained from a mammal or extracts thereof; and blood, saliva, urine, feces, semen, tears, or other body fluids or extracts thereof.

[0098] Inhibition of caspase activity in a biological sample is useful for a variety of purposes that are known to one of skill in the art. Examples of such purposes include, but are not limited to, blood transfusion, organ-transplantation, biological specimen storage, and biological assays.

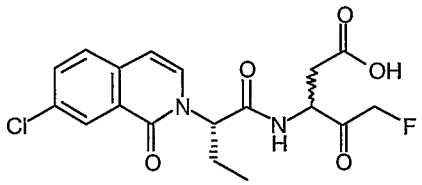
[0099] The compounds of this invention are useful in methods for preserving cells, such as may be needed for an organ transplant or for preserving blood products. Similar uses for caspase inhibitors have been reported (Schierle et al., *Nature Medicine*, **5**, 97 (1999)). The method involves treating the cells or tissue to be

preserved with a solution comprising the caspase inhibitor. The amount of caspase inhibitor needed will depend on the effectiveness of the inhibitor for the given cell type and the length of time required to preserve the cells from apoptotic cell death.

[0100] In order that this invention be more fully understood, the following preparative and testing examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

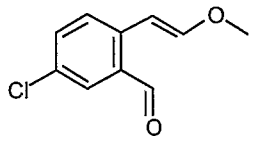
Example 1

(S)-3-[2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



Method A:

5-Chloro-2(2-methoxyvinyl)-benzoic acid

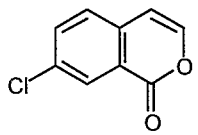


[0101] To a cooled (0°C) slurry of methoxymethyltriphenylphosphonium chloride (39g) in a mixture of diethyl ether (200ml) and *tert*-butanol (50ml) was added potassium *tert*-butoxide (12.8g) portion wise. The resulting mixture was stirred at 0°C for 1 hour, then a solution of 2-formyl-5-chlorobenzoic acid (prepared as

described in *J. Org. Chem.* **61**, 3402 (1996)) (10g) in diethyl ether (50ml) was added dropwise over 15 minutes. The resulting mixture was stirred for 1 hour at 0°C, then warmed to ambient and stirred for an additional 90 minutes. The mixture was diluted with water (200ml) and the organic phase removed. The aqueous phase was acidified to pH 1 with 1M HCl and extracted with ethyl acetate (3x50ml). The combined extracts were washed with brine, dried (magnesium sulfate), filtered and concentrated. The residue was purified by flash chromatography (50% ethyl acetate/hexane) to afford the sub-title compound as a yellow solid (9.13g, 80%). ^{1}H NMR (400MHz,$CDCl_3$) δ 3.70-3.81 (3H, s), 6.20(0.3H, d, *cis* alkene), 6.30 (0.3H, d, *cis* alkene), 6.80(0.7H, d, *trans* alkene), 7.01 (0.7H, d, *trans* alkene), 7.30-8.15 (3H, m) ppm.

Method B:

7-Chloro-isochromen-1-one

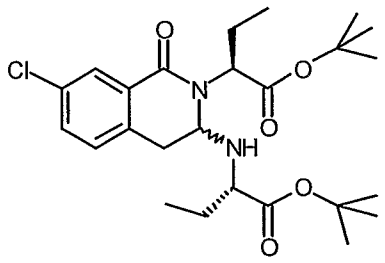


[0102] Concentrated sulphuric acid (15ml) was added to 5-chloro-2(2-methoxyvinyl)-benzoic acid (4.43g) at 0°C. The mixture was stirred for 2 hours, then diluted with ice/water. The product was extracted with ethyl acetate (3x15ml) and the combined extracts washed with saturated sodium bicarbonate solution. The solution was dried (magnesium sulfate), filtered and concentrated. The residue was purified by flash chromatography (0-5% ethyl

acetate/hexane) to afford the sub-title compound as a white solid (3.04g, 81%). mp 109.8-110.9°C. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.51(1H, d), 7.28-7.32(1H, m), 7.41(1H, d), 7.64-7.70(1H, m), 8.28(1H, m) ppm.

Method C:

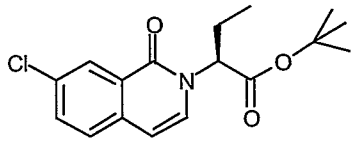
(S)-2-[3-(1-*tert*Butoxycarbonyl-propylamino)-7-chloro-1-oxo-3,4-dihydro-1*H*-isoquinolin-2-yl]-butyric acid *tert*-butyl ester



[0103] A mixture of 7-chloro-isochromen-1-one (10g) and (S)-2-aminobutyric acid, *tert* butyl ester (22g) were heated at 85°C for 24 hours. The mixture was then cooled and purified by flash chromatography (5-25% ethyl acetate/hexane) to afford the sub-title compound as a yellow oil (17.1g, 64%). ^{1}H NMR (400MHz,$CDCl_3$) δ 0.68-1.32 (6H, m), 1.50 (21H, m), 1.92 (1H, m), 2.15 (1H, m), 2.82-3.40 (3H, m), 4.41 (1H, m), 4.68 (1H, m), 7.11 (1H, m),7.35-7.52 (1H, m), 8.05 (1H, m) ppm.

Method D:

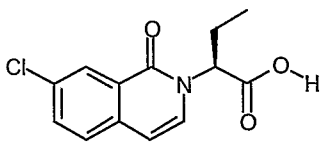
(S)-2-(7-Chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid, *tert* butyl ester



[0104] To a stirred solution of 2-[3-(1-*tert*butoxycarbonyl-propylamino)-7-chloro-1-oxo-3,4-dihydro-1*H*-isoquinolin-2-yl]-butyric acid *tert*-butyl ester (8.58g) in isopropanol (180ml) at 0°C was added concentrated hydrochloric acid (20ml). The resulting mixture was allowed to warm to room temperature and stirred for 18 hours. The mixture was then diluted with ethyl acetate (500ml) and water (150ml). The organic phase was separated and washed with water, then brine, dried (magnesium sulfate), filtered and concentrated. The sub-title product was obtained as a yellow solid (5.57g, 97%). m.p. 111.3-111.8°C; $[\alpha]^{25}_{D}$ -52.3^{0} (c=1, $CHCl_3$); IR (solid) 1731.4, 1649.5, 1593.2, 1229.6, 1152.8, 901.9 cm^{-1}; ^{1}H NMR (400MHz,$CDCl_3$) δ 0.95 (3H, t), 1.48 (9H, s), 1.95 (1H, m), 2.30 (1H, m), 5.55 (1H, m), 6.40 (1H, m), 7.15 (1H, m), 7.49 (1H, m), 7.61 (1H, m), 8.40 (1H, m) ppm; ^{13}C NMR (100MHz,$CDCl_3$) δ 10.9, 24,8, 28.1, 59.2, 82,8, 105.7, 127.3, 127.8, 128.1, 129.5, 133.1, 133.2, 135.4, 161.8, 170.2; MS ES(+) 322.4 (M+H).

Method E:

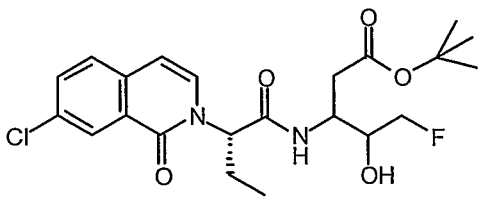
(S)-2-(7-Chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid



[0105] A solution of (S)-2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid, *tert*-butyl ester (322mg) in dichloromethane (14ml) was cooled to 0°C. Trifluoroacetic acid (3.5ml) was added and the resulting mixture allowed to warm to room temperature and stirred for 2 hours. The mixture was then concentrated under reduced pressure and the residue re-dissolved in dichloromethane. This process was repeated several times in order to remove excess trifluoroacetic acid. The resulting solid was slurried in diethyl ether, filtered and washed with more diethyl ether. The solid was then dried to constant weight under vacuum. This gave the sub-title product as a white solid (236mg, 89%). m.p. 159.6-160.1°C; $[\alpha]^{24}_{D}$ -47.0^{0} (c=1.01, $CHCl_3$); IR (solid) 1731.4, 1639.3, 1577.8, 1209.1, 1168.1 cm^{-1}; ^{1}H NMR (400MHz,d_6-DMSO) δ 0.82 (3H, t), 2.00-2.25 (2H, m), 5.20 (1H, m), 6.70 (1H, d), 7.49 (1H, d), 7.70-7.81 (2H, m), 8.18 (1H, s) ppm; ^{13}C NMR (100MHz,d_6-DMSO) δ 10.8, 22.7, 60.8, 104.9, 126.5, 126.6, 128.8, 131.6, 132.5, 133.1, 135.8, 160.5, 171.7; MS ES (+) 266.27 (M+H).

Method F:

(S)-3-[2-(7-Chloro-1-oxo-1*H*-isoquin-2-yl)-butyrylamino]-5-fluoro-4-hydroxy-pentanoic acid *tert*-butyl ester

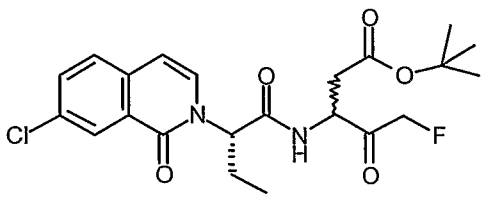


[0106] A stirred mixture of (S)-2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (15g), 3-amino-5-fluoro-4-

hydroxy-pentanoic acid *tert*-butyl ester (prepared as described in *Tetrahedron Lett.* **35**, 9693 (1994)) (12.9g), HOBt (8.4g), DMAP (7.2g) and THF (450ml) was cooled to 0°C then EDC (11.9g) was added. The mixture was allowed to warm to room temperature during 16h then concentrated under reduced pressure. The residue was purified by flash chromatography (30-60% ethyl acetate/hexane) to afford the subtitle compound as a white foam (24.6g, 96%). ^{1}H NMR (400MHz,$CDCl_3$) δ 0.92 (3H, m), 1.13-1.50 (9H, m), 1.95 (1H, m), 2.25 (1H, m), 2.45-2.78 (2H, m), 3.68-4.60 (5H, m), 5.50 (1H, m), 6.60 (1H, m), 7.21-7.60 (4H, m), 8.20-8.31 (1H, m) ppm; ^{19}F NMR (376MHz,$CDCl_3$) (proton decoupled) δ -229.6, -229.7, -230.5, -230.6.

Method G:

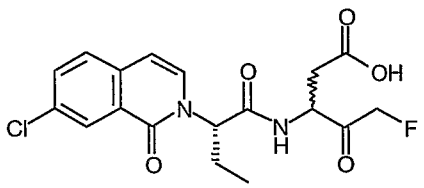
(S)-3-[2-(7-Chloro-1-oxo-1*H*-isoquin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid *tert*-butyl ester



[0107] A stirred solution of 3-[2-(7-chloro-1-oxo-1*H*-isoquin-2-yl)-butyrylamino]-5-fluoro-4-hydroxy-pentanoic acid *tert*-butyl ester (47.8g) in anhydrous DCM (1.2L) was treated with 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (53.5g) at 0°C. The resulting mixture was kept at 0°C for 2 hrs., diluted with ethyl acetate, then poured into a 1:1 mixture of saturated aqueous sodium hydrogen carbonate and saturated aqueous

sodium thiosulfate. The organic layer was removed and the aqueous layer re-extracted with ethyl acetate. The combined organic extracts were dried (magnesium sulfate), filtered and concentrated. The residue was purified by flash chromatography (20-40% ethyl acetate/hexane) to afford the subtitle compound as a white solid (41.9g, 88%). ^{1}H NMR (400MHz,$CDCl_3$) δ 1.00 (3H, t), 1.29 (5H, s), 1.41 (4H, s), 2.01 (1H, m), 2.29 (1H, m), 2.61-3.05 (2H, m), 4.77 (3H, m), 5.50 (1H, m), 6.60 (1H, m), 7.20-7.34 (2H, m), 7.51 (1H, m), 7.62 (1H, m), 8.41 (1H, m) ppm; ^{19}F NMR (376MHz,$CDCl_3$)(proton decoupled) δ -231.89, -232.30.

(S)-3-[2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid

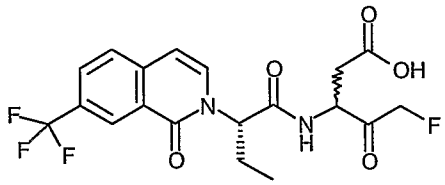


[0108] This was prepared using a procedure similar to that described in method E. The product was isolated as a white solid (98%). IR (solid) 1782.7, 1741.7, 1644.4, 1593.2, 1536.8, 1209.1, 1168.1, 1055.5, 840.4 cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 0.82 (3H, m), 1.81-2.25 (2H, m), 2.25-3.11 (2H, m), 4.15-5.60 (4H, m), 6.70 (1H, m), 7.55 (1H, m), 7.78 (2H, m), 8.15 (1H, s), 8.35-9.00 (1H, brm) ppm; ^{13}C NMR (100MHz, d_6-DMSO) δ 10.6, 23.0, 24.0, 24.6, 32.9, 34.6, 34.7, 47.7, 52.2, 52.3, 58.2, 58.23, 58.7, 59.1, 83.4, 83.5, 85.2, 85.3, 103.9, 104.5, 104.7, 104.8, 126.5, 126.6, 128.8, 131.3, 131.4, 131., 133.1, 135.7, 135.73, 160.8, 170.2, 170.3, 170.4, 172.0, 173.1, 202.6,

202.7; ^{19}F NMR (376MHz, d_6-DMSO) (proton decoupled) δ -226.70, -226.75, -227.51, -230.5, -231.16, -232.61, -232.67, -233.37; MS ES (-) 395.33 (M-H).

Example 2

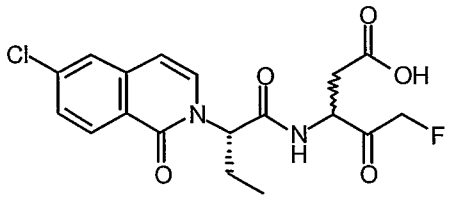
(S)-3-[2-(7-trifluoromethyl -1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



[0109] This was prepared using procedures similar to those described in methods A-G (2-formyl-5-trifluoromethylbenzoic acid was prepared using a procedure similar to that described in *J. Org. Chem.* **61**, 3402 (1996). The product was isolated as a white solid (93% last step). IR (solid) 1782.6, 1746.8, 1644.4, 1629.0, 1603.4, 1321.8, 1275.7, 1168.1, 1127.2, 927.5 cm^{-1}; ^1H NMR (400MHz, d_6-DMSO) δ 0.82 (3H, m), 1.85-2.21 (2H, m), 2.35-3.21 (2H, m), 4.20-5.75 (4H, m), 6.80 (1H, m), 7.68 (1H, m), 7.92 (1H, m), 8.04 (1H, m), 8.49 (1H, s), 8.56-8.95 (1H, brm) ppm; ^{19}F NMR (376MHz, d_6-DMSO) (proton decoupled) δ -61.32, -61.38, -226.70, -226.76, -230.47, -231.06, -232.61, -232.67; MS ES (-) 429.30 (M-H).

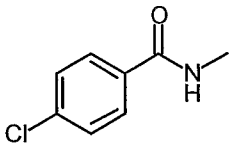
Example 3

(S)-3-[2-(6-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



Method H:

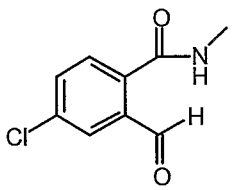
4-Chloro-N-methyl-benzamide



[0110] To a 0°C solution of the 4-chlorobenzoyl chloride (4.50g) in dichloromethane (10mL) was added an 8M solution of methylamine in ethanol dropwise. The solution was stirred for 16 h and then evaporated to dryness. The residue was diluted with saturated sodium bicarbonate solution (10mL) and extracted three times with ethyl acetate (3x20mL), the organics washed with brine (10mL), dried ($MgSO_4$) and concentrated *in vacuo* to afford the sub-title compound as a white solid (4.33g; 97%). ^1H NMR (400MHz, $CDCl_3$) δ 3.00 (3H, s), 7.40 (1H, br) 7.40 (1H, d), 7.70 (1H, d) ppm.

Method I:

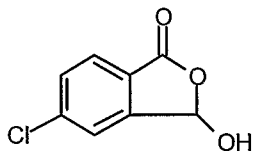
2-formyl-4-chloro-N-methylbenzamide



[0111] To a solution of 4-chloro-N-methyl-benzamide (3.1g) in THF (30mL) was added *n*-butyl lithium (30.1mL of 2.5M hexane solution) and the solution refluxed for 45 min. The solution was then cooled to 0°C and N-methylformanilide (9.27mL) added dropwise over 2 min. The solution was then refluxed for 2 h, then cooled to ambient temperature, water (80mL) added and the solution acidified to pH 1 with 2M HCl. The solution was then extracted three times with ethyl acetate (3x50mL), washed with brine (20mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resulting brown oil was purified on silica by flash chromatography to afford the sub-titled product as a pale yellow solid (2.13g; 59%). ^1H NMR (400MHz, $CDCl_3$) δ 2.90 (3H, s), 4.25 (1H, d, J) 5.60 (1H, d, J), 7.35 (2H, s), 7.60 (1H, s) ppm.

Method J

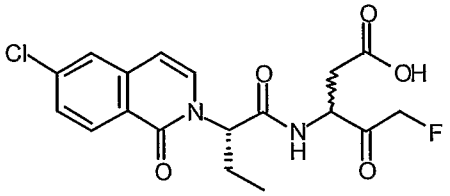
2-Formyl-4-chlorobenzoic acid



[0112] A mixture of 2-formyl-4-chloro-N-methylbenzamide (3.19g) and 10M hydrochloric acid (30ml) was heated at reflux for 18 hours. The mixture was cooled and basified with saturated sodium hydrogen carbonate solution. The solution was then washed with ethyl acetate, then acidified with 2M hydrochloric acid. The product was extracted with ethyl acetate and the combined extracts dried with magnesium sulfate. The solution was then filtered and concentrated. This

furnished 2-formyl-4-chlorobenzoic acid as a yellow solid (2.22g, 75%). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.65 (0.5H, brs), 7.50 (2H, m), 7.65 (1H, m), 7.85 (0.5H, brm), 8.05 (1H, m) ppm.

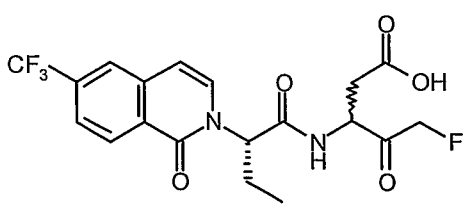
(S)-3-[2-(6-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



[0113] This was prepared from 2-formyl-4-chlorobenzoic acid (prepared as described in methods H-J) using procedures similar to those described in methods A-G. The title compound was isolated by preparative HPLC and was obtained as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (3H, m), 1.90-2.31 (2H, m), 2.65-3.30 (2H, m), 4.20-5.75 (4H, m), 6.65 (1H, m), 7.40-7.60 (3H, m), 8.29 (1H, m), 9.20 (1H, br) ppm; ^{19}F NMR (376 MHz, $CDCl_3$) (proton decoupled) δ -229.80, -232.07, -232.43, -232.58, -232.78; MS ES (-) 395.26 (M-H).

Example 4

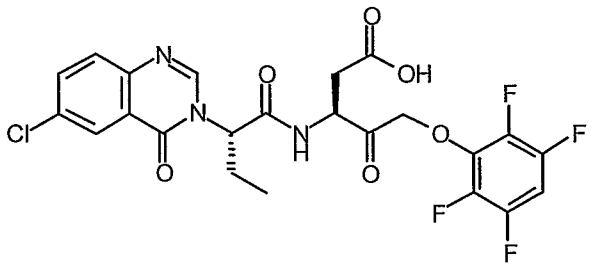
(S)-3-[2-(6-trifluoromethyl -1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



[0114] This was prepared from 2-formyl-4-trifluoromethylbenzoic acid (prepared from 4-trifluoromethylbenzoic acid using methods similar to those described in H-J) using procedures similar to those described in methods A-G. The title compound was isolated as a white solid (95%, last step). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (3H, m), 1.90-2.30 (2H, m), 2.60-3.50 (2H, m), 4.20-5.75 (4H, m), 6.80 (1H, m), 7.50-7.90 (3H, m), 7.92 (1H, m), 8.40-8.60 (1H, m) ppm; ^{19}F NMR (376 MHz,d_6-DMSO) (proton decoupled) δ -63.60, -63.61, -63.65, -231.67, -231.80, -232.06, -232.18; MS ES(+) 431.26 (M+H).

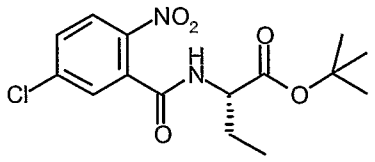
Example 5

(S,S)-3-[2-(6-Chloro-4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)pentanoic acid



Method K:

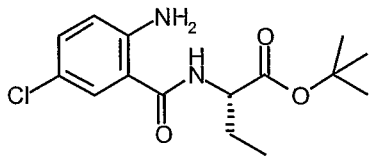
(S)-2-(5-chloro-2-nitro-benzoylamino)-butyric acid-*tert*-butyl ester



[0115] To a stirred solution of 5-chloro-2-nitro-benzoic acid (4.68g) in anhydrous tetrahydrofuran (100ml) at 0°C, was added (S)-2-amino-butyric acid-*tert*-butyl ester hydrochloric acid salt (5g), HOBt (3.45g), DMAP (2.98g), followed by EDC (4.88g) and diisopropylethylamine (3.30g). The reaction mixture was stirred for 10 minutes then warmed to room temperature and stirred for 16 hours before concentration at reduced pressure. The resulting residue was partitioned between EtOAc (100ml) and saturated sodium bicarbonate solution (100ml), the organic layer separated and further washed with 1M hydrochloric acid and saturated brine solution, dried (MgSO4), filtered and concentrated to give the sub-title compound as a pale yellow oil (6.82g, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (1H, d), 7.55 (2H, m), 6.4 (1H, m) 4.7 (1H, m), 2.1 (1H, m), 1.95 (1H, m), 1.5 (9H, s), 1.0 (3H, t) ppm.

Method L:

(S)-2-(2-amino-5- chloro-benzoylamino)-butyric acid-*tert*-butyl ester

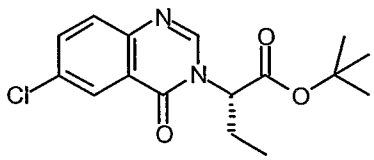


[0116] To a stirred solution of (S)-2-(5-chloro-2-nitro-benzoylamino)-butyric acid-*tert*-butyl ester (5.72g,) in ethanol (250ml) at ambient temperature was added tin(II)chloride *bis*-hydrate (18.8g). The mixture was stirred at ambient for 16 hours. The volume was

reduced to approximately 50ml under reduced pressure, and reaction mixture was then diluted with 1M NaOH until the precipitate formed re-dissolved (400ml). The aqueous was then extracted with diethyl ether (3x100ml). The organic extracts were combined and washed with brine, dried ($MgSO_4$), and concentrated to give the sub-titled compound as a yellow solid (4.60g, 88% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 7.4 (1H, s), 7.2 (1H, d), 6.6 (2H, m), 5.5 (2H, m), 4.6 (1H, m), 2.0 (1H, m), 1.85 (1H, m), 1.5 (9H, s), 0.95 (3H, t) ppm.

Method M:

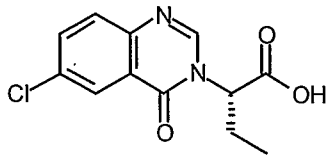
(S)-2-(6-chloro-4-oxo-*4H*-quinazoline-3-yl)-butyric acid-*tert*-butyl ester



[0117] 2-(2-amino-5-chloro-benzoylamino)-butyric acid-*tert*-butyl ester (2.82g) was dissolved in trimethylorthoformate (50ml). Acetic acid (10ml) was added, and reaction mixture refluxed for 48 hours. After this time, the solvents were removed and the residue partitioned between ethyl acetate and saturated sodium bicarbonate solution. The aqueous layer was further extracted with ethyl acetate and the combined organic extracts washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil. This was purified by flash chromatography (20% EtOAc : Petroleum) to give the sub-title compound as an oil (1.23g, 42% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (1H, s), 8.05 (1H,

s), 7.7 (2H, m), 5.3 (1H, m), 2.3 (1H, m), 2.0 (1H, m), 1.5 (9H, s), 1.0 (3H, t) ppm.

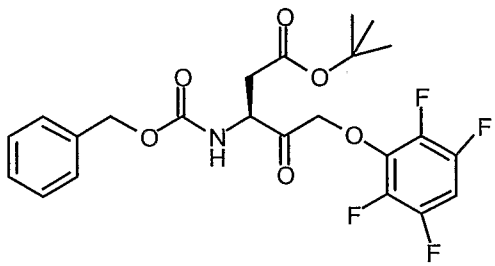
2-(6-chloro-4-oxo-*4H*-quinazoline-3-yl)-butyric acid



[0118] This was prepared using a procedure similar to that described in method E. The sub-title compound was obtained as an off-white solid. ^{1}H NMR (400 MHz, MeOD) δ 8.3 (1H, s), 8.15 (1H, s), 7.8 (1H, d), 7.7 (1H, d), 5.2 (1H, m), 2.4 (1H, m), 2.2 (1H, m), 0.95 (3H, t) ppm.

Method N:

(S)-3-Benzyloxycarbonylamino-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester

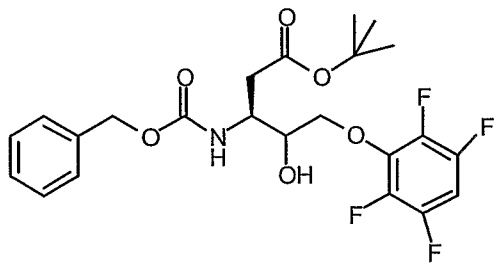


[0119] Potassium fluoride (2.8 g) was added portionwise to a stirred solution of (S)-3-benzyloxycarbonylamino-5-bromo-4-oxo-pentanoic acid *tert*-butyl ester (18.6 g) and 2,3,5,6-tetrafluorophenol (9.3 g) in anhydrous DMF (250 mL) under nitrogen at room temperature. The mixture was then stirred for 18 hours

before being quenched with ethyl acetate and water. The organic layer was removed and washed with sodium bicarbonate solution, dried (magnesium sulfate) and concentrated to give the sub-title product as an off-white solid (21.1 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.76 (1H, dd), 3.06 (1H, dd), 4.67-4.71 (1H, m), 5.12 (1h, d), 5.22 (1H, d), 5.86 (1H, d), 7.35-7.38 (5H, m) ppm; ^{19}F NMR (376 MHz, $CDCl_3$) (proton decoupled) δ -139.98, -140.00, -140.04, -140.06, -157.05, -157.07, -157.11, -157.13; MS ES (+) 486.23 (M+H).

Method O:

(3S)-3-Benzyloxycarbonylamino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester

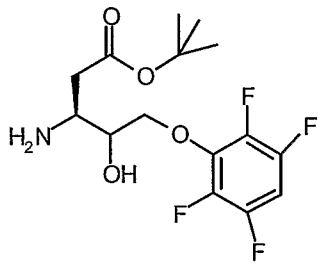


[0120] $NaBH_4$ (1.65 g) was added portionwise to a stirred solution of 3-benzyloxycarbonylamino-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (21.1 g) in anhydrous THF (220 mL) at -20 °C under nitrogen. After stirring at this temperature for 3 hours, the reaction was quenched by the addition of saturated ammonium chloride solution and diluted with DCM. The organic layer was removed and the aqueous layer re-extracted with DCM. The combined organic extracts were washed with brine, dried (magnesium sulfate) and concentrated. The residue was purified by column chromatography (10%-20% ethyl acetate/hexane). The sub-

title compound was obtained as a white solid (14.6 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (9H, s), 2.61-2.77 (2H, m), 3.16-3.36 (1H, 2 x brd d), 4.12-4.22 (2H, m), 4.30-4.33 (1H, m), 5.44-5.69 (1H, 2xd), 6.78-6.86 (1H, m), 7.35-7.36 (5H, m) ppm; ^{19}F NMR (346 MHz, $CDCl_3$) (proton decoupled) δ -139.87, -139.89, -139.93, -139.95, -139.98, -157.02, -157.05, -157.06, -157.08, -157.09, -157.10, -157.12; ES (+) 488.27 (M+H).

Method P:

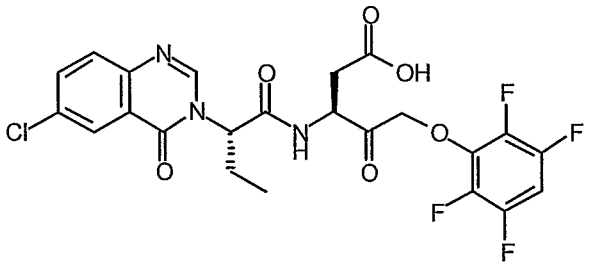
(3S)-3-Amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester



[0121] 10% Pd on carbon (2.92 g) was added portionwise to a solution of 3-benzyloxycarbonylamino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (14.6 g) in anhydrous MeOH (350 mL) which had been degassed under nitrogen (5x). The reaction was further degassed under nitrogen (3x) and hydrogen (5x) and stirred at room temperature for 20 minutes. The palladium catalyst was removed by filtration and the filtrate concentrated to give the sub-titled compound as a white solid (9.5 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (9H, s), 2.35-2.43 (1H, m), 5.67-5.64 (1H, m), 3.37-3.43 (1H, m), 3.77-3.87 (1H, m), 4.28-4.63 (2H, m), 6.77-6.86 (1H, m) ppm; ^{19}F NMR (346 MHz, $CDCl_3$) (proton

decoupled) δ -139.95, -139.97, -140.00, -140.03, -140.05, -140.08, -140.11, -140.13, -157.15, -157.18, -157.21, -157.23, -157.27, -157.29.

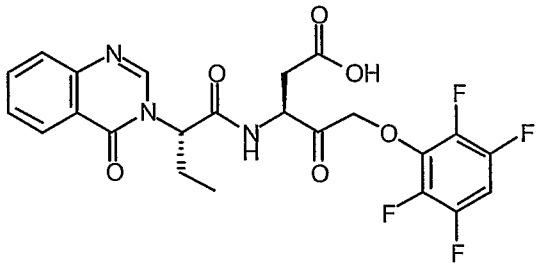
(S,S)-3-[2-(6-Chloro-4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)pentanoic acid



[0122] This compound was prepared using procedures similar to those described in methods F, G, and E. The title compound was obtained as a white solid (TFA salt)(94%, last step). IR (solid) 3298.7, 1741.4, 1691.4, 1660.3, 1601.1, 1517.4, 1490.8, 1320.6, 1244.7, 1174.5, 1104.4, 939.2, 836.5, 715.5, 666.8 and 656.0 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (1H, m), 8.4 (1H, s), 8.05-7.5 (4H, m), 5.45-5.15 (3H, m), 4.6 (1H, m), 2.25-2.0 (2H, m) and 0.9-0.75 (3H, m) ppm; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -140.57, -140.59, -140.63, -140.65, -141.02, -141.04, -141.08, -141.10, -156.75, -156.77, -156.81, -156.83, -156.94, -156.96, -157.00, -157.02; MS ES (+) 544.2 (M+H).

Example 6

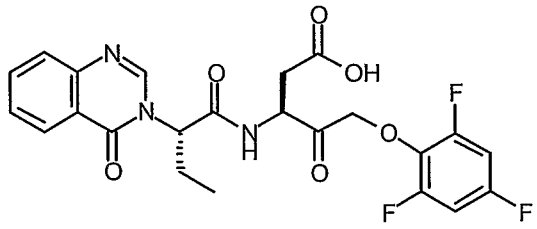
(S,S)-4-Oxo-3-[2-(4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-5-(2,3,5,6-tetrafluoro-phenoxy)pentanoic acid



[0123] This compound was prepared using 2-(4-oxo-*4H*-quinazoline-3-yl)-butyric acid (synthesized from 2-nitrobenzoic acid using procedures similar to those described in methods K-M and E) and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The title compound was obtained as a white solid (TFA salt) (85%, last step). IR (solid) 1792.8, 1726.3, 1669.9, 1521.4, 1485.6, 1183.5, 1142.5, 1091.3 and 932.5 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.0-8.65 (1H, m), 8.35 (1H, s), 8.1 (1H, m), 7.8 (1H, m), 7.65 (1H, m), 7.55 (2H, m), 5.4-4.3 (4H, m), 2.8-2.5 (2H, m), 2.2-2.0 (2H, m) and 0.9-0.7 (3H, m) ppm; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -140.57, -140.60, -140.63, -140.66, -141.03, -141.05, -141.09, -141.11, -156.77, -156.80, -156.83, -156.86, -156.94, -156.97, -157.00, -157.03; MS ES(+) 510.26 (M+H).

Example 7

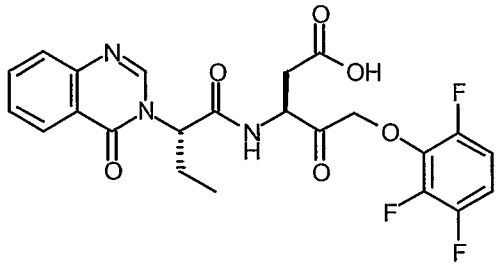
(S,S)-4-Oxo-3-[2-(4-oxo-4H-quinazolin-3-yl)-butyrylamino]-5-(2,4,6-trifluoro-phenoxy)-pentanoic acid



[0124] This compound was prepared using 2-(4-oxo-*4H*-quinazoline-3-yl)-butyric acid (synthesized from 2-nitrobenzoic acid using procedures similar to those described in methods K-M and E) and (3S)-3-amino-4-hydroxy-5-(2,4,6-trifluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared from 2,4,6-trifluorophenol using procedures similar to those described in methods N-P) using procedures similar to those described in methods F, G and E. The title compound was obtained as a white solid (TFA salt) (71%, last step). IR (solid) 3308.9, 3075.4, 2928.4, 1803.1, 1726.9, 1672.7, 1612.1, 1510.0, 1478.7, 1452.8, 1407.6, 1231.3, 1199.3, 1121.0, 1040.4, 997.6, 933.1, 839.7, 772.9, 720.3 and 698.4 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.84-0.86 (3H, m), 2.04-2.95 (4H,), 4.05-5.48 (5H, 6), 7.13-7.21 (2H, m), 7.54-7.57 (1H, m), 7.69-7.72 (1H, m), 7.84-7.88 (1H, m), 8.07-8.13 (1H, m), 8.38 (1H, s), 8.60-8.97 (1H, 2xdd) ppm; ^{19}F NMR (376 MHz, DMSO-d_6) (proton decoupled) δ -115.4, -115.6, -124.9, -125.0, -125.6; MS ES (+): 492.3 (M+H).

Example 8

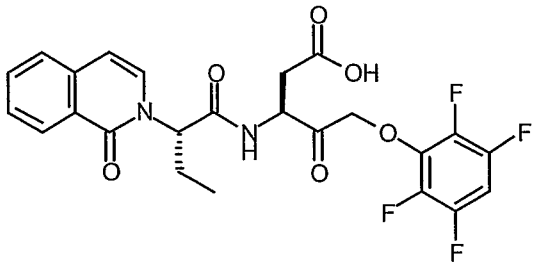
(S,S)-4-Oxo-3-[2-(4-oxo-4H-quinazolin-3-yl)-butyrylamino]-5-(2,5,6-trifluoro-phenoxy)-pentanoic acid



[0125] This compound was prepared using 2-(4-oxo-*4H*-quinazoline-3-yl)-butyric acid (synthesized from 2-nitrobenzoic acid using procedures similar to those described in methods K-M and E) and (3S)-3-amino-4-hydroxy-5-(2,3,6-trifluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared from 2,3,6-trifluorophenol using procedures similar to those described in methods N-P) using procedures similar to those described in methods F, G and E. The title compound was obtained as a white solid (TFA salt)(87%, last step). IR (solid) 3308.9, 3075.4, 2928.4, 1803.1, 1726.9, 1672.7, 1612.1, 1510.0, 1478.7, 1452.8, 1407.6, 1231.3, 1199.3, 1121.0, 1040.4, 997.6, 933.1, 839.7, 772.9, 720.3 and 698.4 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ0.84-0.86 (3H, m,), 2.04-2.95 (4H, 3 m), 4.05-5.48 (5H, 6 x m), 7.13-7.21 (2H, m,), 7.54-7.57 (1H, m), 7.69-7.72 (1H, m), 7.84-7.88 (1H, m), 8.07-8.13 (1H, m), 8.38 (1H, s), 8.60-8.97 (1H, 2) ppm; ^{19}F NMR (376MHz, DMSO) (proton decoupled) δ -115.4, -115.6, -124.9, -125.0, -125.6; MS ES (+):492.3 (M+H).

Example 9

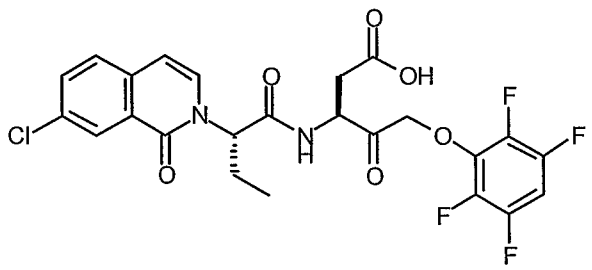
(S,S)-3-[2-(1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid



[0126] This compound was prepared using (S)-2-(1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (prepared from 2-formylbenzoic acid using procedures similar to those described in methods A-E) and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The title compound was isolated by preparative HPLC. IR (solid) 2960.2, 1780.1, 1746.2, 1646.6, 1619.0, 1589.1, 1517.4, 1490.3, 1427.8, 1260.3, 1206.2, 1176.3, 1098.9, 938.4, 788.1, 748.3, 714.2, 692.5, 665.3 and 655.8 cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6) δ 0.78-0.83 (3H, m), 1.89-1.96 (1H, m), 2.08-2.13 (1H, m), 2.50-2.78 (2H, m), 4.35-5.45 (4H, 3xm), 6.64-6.69 (1H, m), 7.74-7.73 (5H, m), 8.18-8.20 (1H, m), 8.81 & 8.90 (1H, d) ppm; ^{19}F NMR (376MHz, DMSO)(proton decoupled) δ -141.01, -141.03, -141.07, -141.09, -156.80, -156.82, - 156.86, -156.88; MS ES (+) 509.2 (M+H).

Example 10

(S,S)-3-[2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid

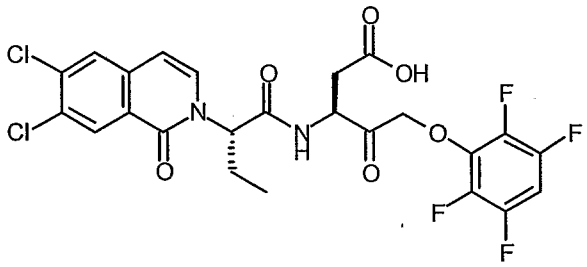


[0127] This compound was prepared using (S)-2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (prepared as described in methods A-E) and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The product was isolated as a white solid (94% last step). IR (solid) 1639.3, 1618.8, 1593.2, 1516.4, 1485.6, 1219.4, 1168.1, 1106.7, 932.6, 830.2 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.80 (3H, t), 1.94-2.12 (2H, m), 2.55-2.61 (1H, m), 2.74-2.80 (1H, m), 4.58-4.63 (1H, m), 5.12-5.76 (3H, m), 6.70 (1H, d), 7.51-7.78 (4H, m), 8.11-8.12 (1H, m), 8.60-8.95 (1H,2xd) ppm; ^{13}C NMR (100 MHz, d_6-DMSO) δ 23.8, 24.5, 32.9, 34.6, 47.8, 52.8, 55.2, 58.2, 58.9, 74.4, 75.6, 100.1, 100.3, 100.5, 101.0, 101.2, 104.6, 126.5, 136.6, 131.3, 131.4, 133.0, 135.6, 135.6, 139.0, 139.1, 141.4, 141.6, 144.6, 144.8, 144.9, 147.1, 147.1, 160.7, 170.4, 172.0, 173.0, 202.2; ^{19}F NMR (376 MHz,d_6-DMSO) (proton decoupled) δ -140.57, -140.60, -140.64, -140.66, -141.00, -141.03, -141.06, -141.09, -

156.78, -156.80, -156.84, -156.86, -156.96, -156.98, -157.02, -157.04; MS ES(+) 543.20 (M+H).

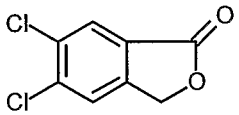
Example 11

(S,S)-3-[2-(6,7-dichloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid



Method Q:

5,6-Dichloro-3*H*-isobenzofuran-1-one

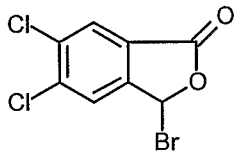


[0128] $NaBH_4$ (5.2 g) was added to a stirred solution of 4,5-dichlorophthalic anhydride (20 g) in anhydrous DMF (100 mL) at 0°C under nitrogen in small portions over 1 hour. The reaction was warmed to room temperature for a further 2 hours and poured into ice/1M HCl. The resultant white precipitate (4,5-dichloro-2-hydroxymethyl-benzoic acid) was collected by filtration and dried under vacuum. The precipitate was suspended in toluene (200 mL) with catalytic p-toluenesulfonic acid and heated to reflux under Dean-Stark conditions (precipitate dissolves on heating) for 18 hours. The reaction was cooled to room temperature and the resultant

white precipitate collected by filtration to give the sub-title compound as a white solid (14.0 g, 75%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 5.40 (2H, s), 8.05 (1H, s), 8.15 (1H, s) ppm.

Method R:

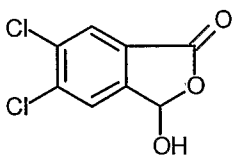
3-Bromo-5,6-dichloro-3*H*-isobenzofuran-1-one



[0129] A suspension of 5,6-Dichloro-3*H*-isobenzofuran-1-one (1.45 g), N-bromosuccinamide (1.27 g) and catalytic benzoyl peroxide in chloroform (30 mL) was heated to reflux for 1 hour. After cooling, the reaction mixture was washed with water, brine, dried (magnesium sulfate), filtered and concentrated to give the sub-title compound as a white solid (1.82 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (1H, s), 7.77 (1H, s), 8.03 (1H, s) ppm.

Method S:

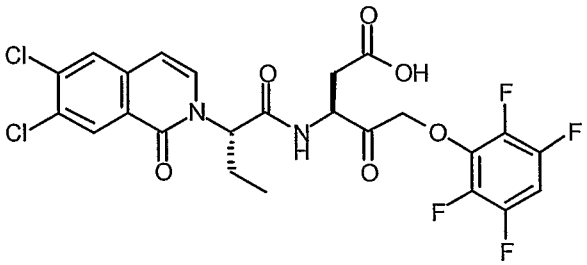
4,5-Dichloro-2-formyl-benzoic acid



[0130] A suspension of 3-bromo-5,6-dichloro-3*H*-isobenzofuran-1-one (2.0 g) in 5% aqueous HCl (10 mL) and 80% aqueous dioxane (25 mL) was heated to reflux for 2 hours. The solvent was removed and the resulting residue

re-dissolved in ethyl acetate, dried (magnesium sulfate) and concentrated. The resultant yellow solid was recrystallized from DCM/hexane to give the sub-title compound as a white solid (1.13 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (0.84H, s), 7.95 (0.16H, s), 8.05 (0.84H), 8.12 (0.16H, s), 8.14 (0.84H, s), 8.41 (0.84H,), 10.41 (0.16H, s), 11.07 (0.16 H, brd s) ppm.

(S,S)-3-[2-(6,7-dichloro-1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid

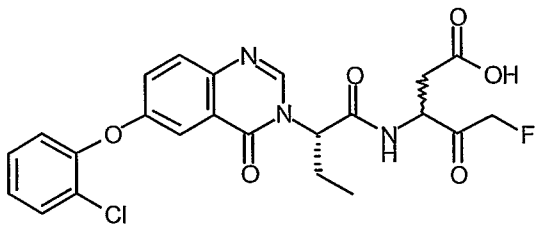


[0131] This compound was prepared using (S)-2-(6,7-dichloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (synthesized from 4,5-dichloro-2-formyl-benzoic acid [prepared as described in methods Q-S] using procedures similar to those described in methods A-E) and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The title compound was isolated as a white solid (94% last step). IR (solid) 1784.5, 1734.7, 1650.1, 1610.2, 1585.4, 1515.7, 1490.8, 1426.0, 1216.9, 1172.1, 1092.5, 933.1 cm^{-1}; ^{1}H NMR (400 MHz,d_6-DMSO) δ 0.80 (3H, t), 1.90-1.98 (1H, m), 2.04-2.12 (1H, m), 2.55-2.79 (2H, m), 4.56-4.71 (1H, m), 5.08-5.41 (3H, m), 6.67 (1H,

d), 7.56-7.59 (2H, m), 8.07 (1H, brd s), 8.25 (1H, d), 8.85-8.95 (1H, 2 x d), 12.73 (1H, brd s) ppm; ^{19}F NMR (376 MHz, d_6-DMSO) (proton decoupled) δ -140.93, -140.95, -140.99, -141.01, -141.04, -141.07, -141.10, -156.76, -156.79, -156.82, -156.85, -156.89, -156.91; MS ES (+): 577.14 (M+H).

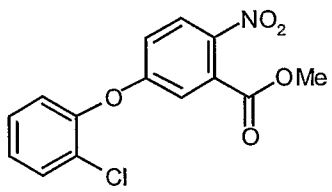
Example 12

3-[(2S)-2-[6-(2-Chloro-phenoxy)-4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



Method T:

2-Nitro-5-(2-chlorophenoxy)methyl benzoate

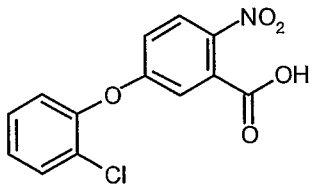


[0132] To a stirred solution of 5-chloro-2-nitrobenzoic acid methyl ester (1g, 4.6 mmol) in dimethylformamide (15ml), was added potassium carbonate (0.96g), and 2-chlorophenol (0.66g). The reaction mixture was stirred at 90°C for 16 hours. After this time the reaction mixture was poured into ethyl acetate (50ml) and washed with water (50ml), brine (50ml), dried ($MgSO_4$), and evaporated. The residue was purified by flash chromatography (1:9 ethyl acetate: pet ether) to give the

sub-titled compound as a yellow oil (1.22g, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (1H, m), 7.6-7.0 (6H, m), 3.95 (3H, s) ppm.

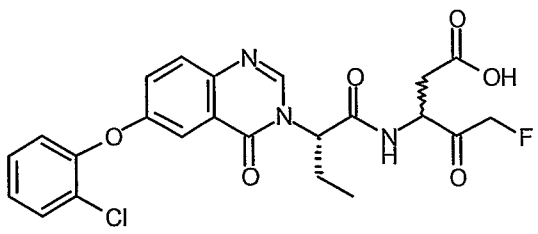
Method U:

2-Nitro-5-(2-chlorophenoxy)benzoic acid



[0133] To a stirred solution of 2-nitro-5-(2-chlorophenoxy)methyl benzoate (1.22g) in THF (8 ml) was added lithium hydroxide (0.333g) in water (2ml). The reaction mixture was stirred at ambient temperature for 16 hours. After this time the reaction mixture was acidified with aqueous 1M HCl. The aqueous layer was extracted with ethyl acetate (2 x 50 ml). The organics were combined and washed with saturated brine solution and dried ($MgSO_4$). The solvents were removed under vacuum to give the sub-title compound as an off-white solid (0.63g, 54% yield). ^{1}H NMR (400 MHz, MeOD) δ 8.05-7.0 (7H, m) ppm.

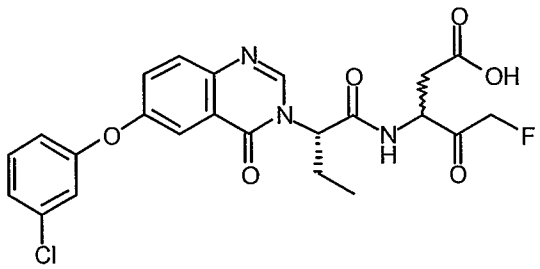
3-[(2S)-2-[6-(2-Chloro-phenoxy)-4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



[0134] This compound was prepared from 2-(6-[2-chlorophenoxy]-4-oxo-*4H*-quinazoline-3-yl)-butyric acid, (synthesized from 5-chloro-2-nitrobenzoic acid methyl ester and 2-chlorophenol as described in methods T-U, K-M and E) using procedures similar to those described in methods F, G and E. The title compound was obtained as a white solid (TFA salt) (82%, last step). IR (solid) 1783.38, 1721.55, 1664.48, 1550.32, 1498.00, 1474.22, 1264.94, 1193.60, 1136.52, 1055.66 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.81 (3H, m), 1.95-2.26 (2H, m), 2.50-2.96 (2H, m), 4.20-4.70 (1.5H, m), 5.04-5.46 (2.5H, m), 7.26-7.39 (3H, m), 7.45 (1H, m), 7.57-7.70 (2H, m), 7.78 (1H, m), 8.35 (1H, m), 8.63-8.98 (1H, brm) ppm; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -226.73, -226.78, -230.43, -230.90, -232.64, -232.55; MS ES (+) 490.40 (M+H).

Example 13

3-[(2S)-2-[6-(3-Chlorophenoxy)-4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid

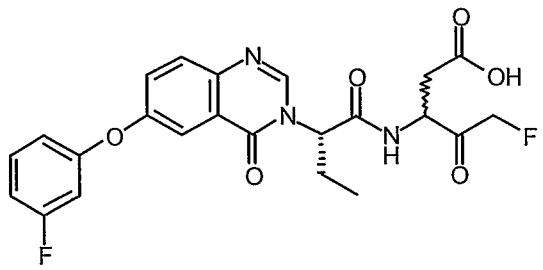


[0135] This compound was prepared from 2-(6-[3-chlorophenoxy]-4-oxo-*4H*-quinazoline-3-yl)-butyric acid, (synthesized from 5-chloro-2-nitrobenzoic acid methyl ester and 3-chlorophenol using methods similar to those described in T-U, K-M and E) using procedures similar to

those described in methods F, G and E. The product was obtained as a white solid (TFA salt) (85%, last step). IR (solid) 1716.79, 1673.99, 1583.62, 1469.46, 1279.21, 1198.35, 1136.52 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75-0.9 (3H, m), 1.95-2.2 (2H, m), 2.5-2.9 (2H, m), 4.2-4.7 (2H, m), 5.05-5.45 (2H, m), 7.1 (1H, m), 7.25 (1H, m), 7.3 (1H, m), 7.45 (1H, m), 7.55 (1H, m), 7.6 (1H, m), 7.8 (1H, m), 8.35 (1H, m), and 8.65-9.0 (1H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.6, 202.5, 173.1, 172.0, 172.0, 170.0, 169.8, 169.8, 160.2, 160.1, 158.8, 157.4, 155.3, 144.1, 134.5, 132.1, 130.1, 126.6, 124.6, 122.6, 122.5, 119.7, 118.2, 113.7, 85.3, 85.2, 83.5, 83.4, 82.9, 58.2, 57.8, 57.5, 53.0, 52.3, 52.2, 47.8, 34.6, 33.0, 24.4, 23.8 and 10.66; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -226.72, -226.78, -230.90 and-232.65; MS ES(+) 490.25 (M+H).

Example 14

5-Fluoro-3-{(2S)-2[6-(3-fluorophenoxy)-4-oxo-4*H*-quinazolin-3-yl]-butyrylamino}-4-oxo-pentanoic acid

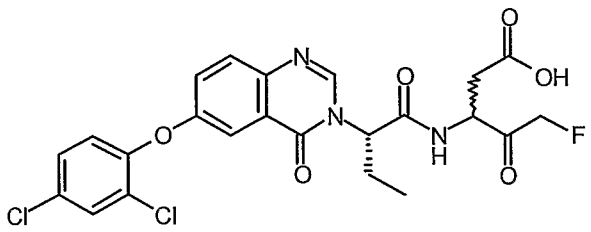


[0136] This compound was prepared from 2-[6-(3-fluorophenoxy)-4-oxo-4*H*-quinazolin-3-yl]-butyric acid, (synthesized from 5-chloro-2-nitrobenzoic acid methyl ester and 3-fluorophenol using methods similar to those described in T-U, K-M and E) using procedures similar to

those described in methods F, G and E. The product was obtained as a white solid (TFA salt) (95%, last step). IR (solid) 1598.20, 1274.17, 1178.86, 1159.80, 1116.92 and 954.90 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.8-0.95 (3H, m), 1.95-2.2 (2H, m), 2.55-2.95 (2H, m), 4.2-4.7 (2H, m), 5.05-5.5 (2H, m), 6.95(1H, m), 7.05 (1H, m), 7.45 (1H, m), 7.55 (1H, m), 7.6 (1H, m), 7.8(1H, m), 8.4(1H, m), and 8.65-9.0 (1H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.0, 169.9, 169.8, 164.5, 162.1, 160.2, 155.3, 144.1, 132.0, 131.9, 130.0, 126.9, 122.6, 115.5, 113.7, 111.5, 111.3, 107.4, 107.1, 85.3, 58.2, 57.8, 52.4, 52.2, 34.6, 23.8, and 10.7; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -110.84, -226.73, -226.79, -230.41, -230.91, -232.64 and -232.66; MS ES(+) 474.29 (M+H).

Example 15

3-{(2S)-2-[6-(2,4-Dichloro-phenoxy)-4-oxo-4*H*-quinazolin-3-yl]-butyrylamino}-5-fluoro-4-oxo-pentanoic acid

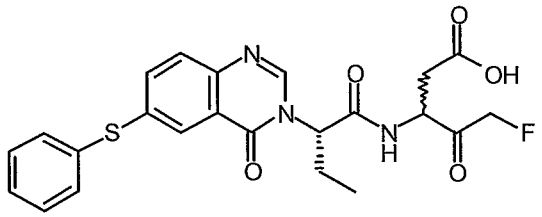


[0137] This compound was prepared from 2-[6-(2,4-dichlorophenoxy)-4-oxo-4*H*-quinazolin-3-yl]-butyric acid, (synthesized from 2-nitro-5-2,4-dichlorophenoxybenzoic acid methyl ester using methods similar to those described in U, K-M and E) using procedures similar to those described in methods F, G and E. The product was obtained as a white solid (TFA salt)(96 %, last step). IR (solid) 1778.63, 1716.79, 1669.23, 1493.25, 1469.46,

1264.94, 1198.35, 1150.79, 1098.47 and 1055.66 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 0.60-1.05 (3H, m), 1.94-2.26 (2H, m), 2.26-2.95 (2H, m), 4.15-4.75 (2H, m), 4.76-5.95 (2H, m), 7.25-7.45 (2H, m), 7.50 (1H, m), 7.65 (1H, m), 7,80 (1H, m), 7.87 (1H, m), 8.36 (1H, s), 8.60-9.01 (1H, brm) ppm; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -226.72, -226.77, -230.41, -230.88, -232.65 ; MS ES (+) 524.34 (M+H).

Example 16

5-Fluoro-4-oxo-3-[(2S)-2-(4-oxo-6-phenylsulfanyl-4*H*-quinazolin-3-yl)-butyrylamino]-pentanoic acid

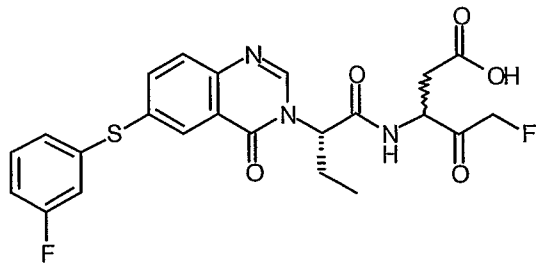


[0138] This compound was prepared from (S)-2(4-oxo-6-phenylsulfanyl-4*H*-quinazolin-3yl)-butyric acid, (synthesized from 5-chloro-2-nitrobenzoic acid methyl ester and thiophenol using methods similar to those described in T-U, K-M and E) using procedures similar to those described in methods F, G and E. The product was obtained as a white solid (TFA salt)(81%, last step). IR (solid) 1791.61, 1720.53, 1668.40, 1602.06, 1545.20, 1474.12, 1194.53, 1142.41 and 1057.11 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.6-8.6 (1H, m), 8.3 (1H, s), 7.85 (1H, s), 7.75-7.6 (2H, m), 7.45-7.35 (5H, m), 5.4-5.0 (2H, m), 4.6-4.15 (2H, m), 2.85-2.35 (2H, m), 2.2-1.95 (2H, m) and 0.85-0.7 (3H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 202.6, 202.5, 173.1, 172.0, 172.0, 169.9, 169.8, 160.0, 146.5,

136.1, 135.4, 133.6, 133.3, 130.3, 128.8, 128.7, 126.7, 126.6, 122.2, 122.2, 104.0, 85.3, 85.2, 83.5, 83.4, 58.3, 57.9, 57.4, 52.3, 52.2, 47.8, 34.6, 33.0, 24.4, 23.9, 23.8 and 10.6; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -226.72, -226.78, -230.41, -231.92, -232.65 and -232.68; MS ES(+) 472.27 (M+H).

Example 17

5-Fluoro-3{(2S)-2[6-(3-fluoro-phenylsulfanyl)-4-oxo-4*H*-quinazolin-3-yl]-butyrylamino}-4-oxo-pentanoic acid

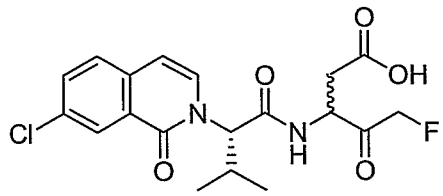


[0139] This compound was prepared from (S)-2[6-(3-fluoro-phenylsulfanyl)-4-oxo-4*H*-quinazolin-3-yl]-butyric acid (synthesized from 5-chloro-2-nitrobenzoic acid methyl ester and 3-fluorothiophenol using methods similar to those described in T-U, K-M and E) using procedures similar to those described in methods F, G and E. The product was obtained as a white solid (TFA salt)(95 %, last step). IR (solid) 1779.2, 1722.0, 1660.1, 1602.9, 1574.3, 1474.3, 1178.8 and 1059.7 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (3H, m), 1.92-2.25 (2H, m), 2.41-2.92 (2H, m), 4.20-4.85 (1.5H, m), 5.01-5.50 (2.5H, m), 7.12-7.27 (3H, m), 7.40-7.50 (1H, m), 7.70-7.86 (2H, m), 8.03 (1H, m), 8.43 (1H, m), 8.65-9.02 (1H, brm) ppm; ^{19}F NMR (376 MHz, DMSO-d_6)(proton decoupled) δ -111.83, -226.73, -

226.79, -230.40, -230.91, -232.65, -232.67; MS ES (+) 490.35 (M+H).

Example 18

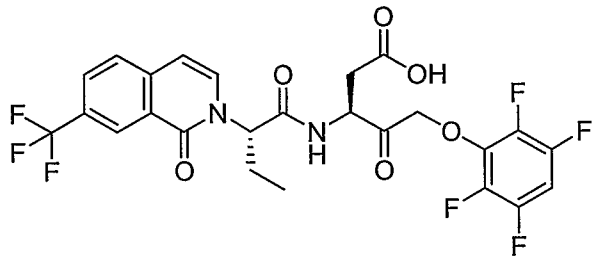
(S)-3-[2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-3-methylbutyrylamino]-5-fluoro-4-oxo-pentanoic acid



[0140] This was prepared using procedures similar to those described in methods A-G (S-valine *tert*-butyl ester was used in method C). The product was isolated as a white solid (91% last step). IR (solid) 1777.5, 1644.4, 1608.5, 1588.1, 1541.9, 1198.9, 1168.1, 1045.2, 830.2 cm^{-1}; ^{1}H NMR (400MHz, d_6-DMSO) δ 0.70 (3H, m), 0.89-1.14 (3H, m), 2.31 (1H, m), 2.45-2.98 (2H, m), 4.05-4.75 (1.6H, m), 4.97-5.40 (2.4H, m), 6.70 (1H, m), 7.60-7.95 (3H, m), 8.18 (1H, m), 8.75-9.21 (1H, m) ppm; ^{19}F NMR (376MHz, d_6-DMSO) (proton decoupled) δ -226.28, -226.71, -230.58, -230.64, -231.75, -232.32; MS ES (+) 411.36 (M+H).

Example 19

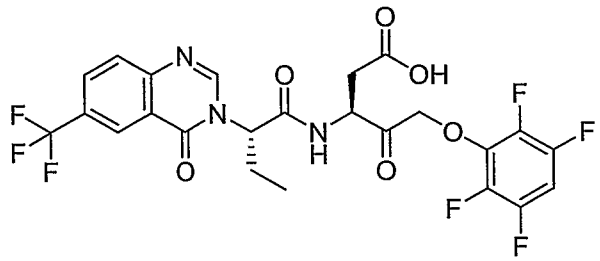
(S,S)-3-[2-(7-trifluoromethyl -1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid



[0141] This compound was prepared using (S)-2-(7-trifluoromethyl -1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (prepared as described in methods A-E, see example 2) and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The product was isolated as a white solid (90% last step). IR (solid) 1791.6, 1649.5, 1516.8, 1493.1, 1322.5 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ 0.85 (3H, t), 1.90-2.20 (2H, 2 x m), 2.50-2.90 (2H, 2 x m), 4.70 (1H, m), 5.25 (2H, dd), 5.45 (1H, m), 6.80 (1H, d), 7.65 (1H, m), 7.70 (1H, d), 7.95 (1H, d), 8.05 (1H, d), 8.45 (1H, s), 9.00 (1H, d) ppm; ^{19}F NMR (376 MHz,d_6-DMSO) (proton decoupled) δ -56.60, -70.15, -136.38, -152.08.

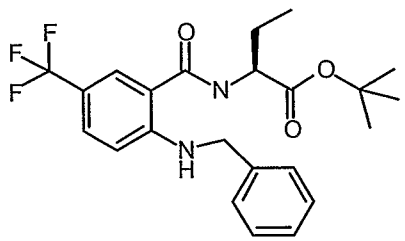
Example 20

(S,S)-3-[2-(7-trifluoromethyl -4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)pentanoic acid



Method V:

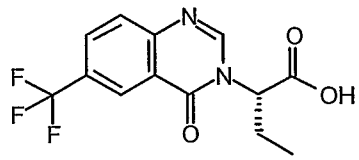
(S)-2-(2-Benzylamino-5-trifluoromethyl-benzoylamino)-butyric acid *tert*-butyl ester



[0142] To a solution of (S)-2-(2-chloro-5-trifluoromethyl-benzoylamino)-butyric acid *tert*-butyl ester (prepared from 2-chloro5-trifluoromethylbenzoic acid and (S)-2-aminobutyric acid, *tert* butyl ester in a procedure similar to that described in method F) (3g) and benzylamine (1.1ml) in N-methylpyrolidine (40ml) was added potassium carbonate (1.6g), copper (30mg) and copper (I) bromide (15mg). The mixture was heated at 160°C for 3 hours then cooled to room temp. and diluted with saturated ammonium chloride solution. The mixture was extracted with ethyl acetate and the organic solution

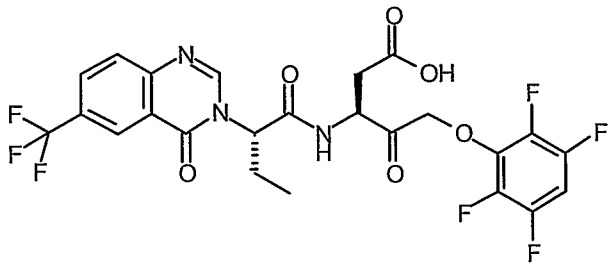
dried (magnesium sulfate), filtered and concentrated. The residue was purified on silica gel, eluting with 15% ethyl acetate/hexane. The sub-titled product was obtained as a white solid (950mg, 27%). ^{1}H NMR (400 MHz,$CDCl_3$) δ 1.04 (3H, t), 1.50 (9H, s), 2.86 (1H, m), 2.02 (1H, m), 4.50 (2H, d), 4.65 (1H, m), 6.69 (2H, m), 7.21-7.70 (7H, m), 8.42 (1H, m) ppm; MS ES(-) 435.32 (M-H).

(S)-2-(6-trifluoromethyl -4-oxo-*4H*-quinazoline-3-yl)-butyric acid



[0143] This compound was prepared from (S)-2-(2-benzylamino-5-trifluoromethyl-benzoylamino)-butyric acid *tert*-butyl ester using procedures similar to those described in methods P, M and E. The sub-title compound was as a white solid. ^{1}H NMR (400 MHz,d_6-DMSO) δ 0.88 (3H, t), 2.22 (2H, m), 5.15 (1H, m), 7.95 (1H, m), 8.20 (1H, m), 8.40 (1H, s), 8.60 (1H, s) ppm; MS ES (+) 301.15 (M+H).

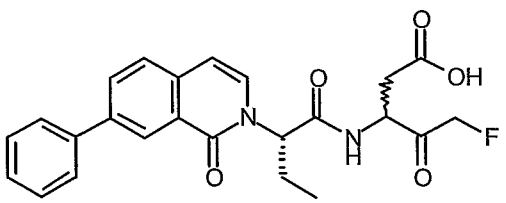
(S,S)-3-[2-(7-trifluoromethyl -4-oxo-4*H*-quinazolin-3-yl)-butyrylamino]-4-oxo-5-(2,3,5,6-tetrafluoro-phenoxy)pentanoic acid



[0144] This compound was prepared from 2-(6-trifluoromethyl -4-oxo-*4H*-quinazoline-3-yl)-butyric acid and (3S)-3-amino-4-hydroxy-5-(2,3,5,6-tetrafluoro-phenoxy)-pentanoic acid *tert*-butyl ester (prepared as described in methods N-P) using procedures similar to those described in methods F, G and E. The titled compound was obtained as a white solid (TFA salt) (90% last step). IR (solid) 1690, 1518, 1490, 1317, 1254, 1170, 1128 cm^{-1}; ^1H NMR (400 MHz, d_6-DMSO) δ0.8-0.9 (3H, m), 2.05-2.2 (2H, m), 2.57-2.80 (2H, m), 4.55-4.62 (2H, m), 5.20-5.35 (3H, m), 7.50-7.62 (1H, m), 7.95 (1H, d), 8.20(1H, d), 8.38 (1H, m), 8.52 (1H, s), 9.04 (1H, d) ppm; MS ES(+) 578.2 (M+H).

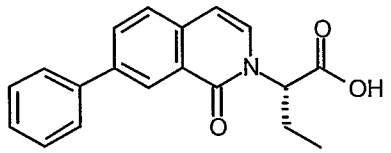
Example 21

(S)-3-[2-(7-phenyl -1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



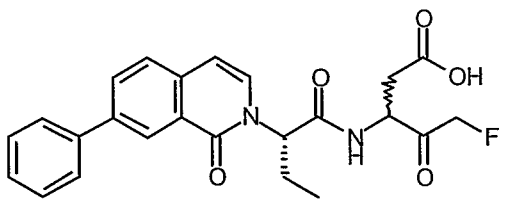
Method W:

(S)-2-(7-phenyl-1-oxo-1H-isoquinolin-2-yl)-butyric acid



[0145] To a solution of (S)-2-(7-chloro-1-oxo-1*H*-isoquinolin-2-yl)-butyric acid (prepared as described in methods A-E) (200mg) and phenyl boronic acid (276mg) in THF (2ml) was added palladium (II) acetate (1.7mg), 2-(di-*tert*-butylphosphino)biphenyl (4.5mg) and potassium fluoride (262mg). The resulting mixture was heated at reflux for 18 hours, then cooled and diluted with 1M HCl (5ml) and ethyl acetate (15ml). The organic phase was separated and the aqueous extracted with ethyl acetate (2x15ml). The combined organics were dried (magnesium sulfate), filtered and concentrated. The residue was purified through a plug of silica gel, then further purified by radial chromatography eluting with 1% acetic acid in ethyl acetate. This gave the subtitled product as a brown solid (153mg, 66%). ^{1}H NMR (400 MHz,$CDCl_3$) δ 1.00 (3H, t), 2.15 (1H, m), 2.40 (1H, m), 5.37 (1H, m), 6.70 (1H, d), 7.15 (1H, d), 7.45 (1H, m), 7.55 (2H, m), 7.68 (1H, m), 7.75 (2H, m), 7.99 (1H, d), 8.70 (1H, s) ppm.

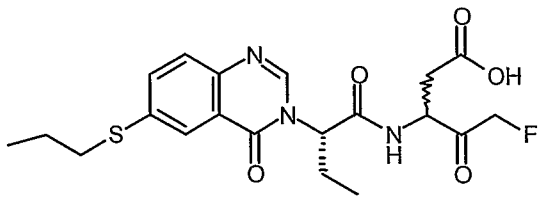
(S)-3-[2-(7-phenyl -1-oxo-1*H*-isoquinolin-2-yl)-butyrylamino]-5-fluoro-4-oxo-pentanoic acid



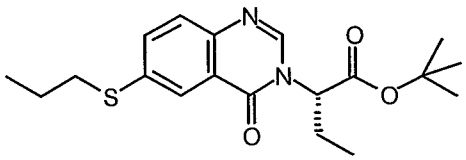
[0146] This compound was prepared from (S)-2-(7-phenyl-1-oxo-1H-isoquinolin-2-yl)-butyric acid using procedures similar to those described in methods F, G and E. The titled compound was obtained as a white solid (58% last step). ^{1}H NMR (400 MHz, d_6-DMSO) δ8.95-8.5 (1H, m), 8.45 (1H, s), 8.05 (1H, m), 7.8-7.7 (2H, m), 7.65-7.35 (4H, m), 6.7-6.65 (1H, m), 5.6-5.0 (2.4H, m), 4.7-4.2 91.6H, m), 2.9-2.3 (H, m), 2.15-1.85 (2H, m), 0.8-0.7 (3H, m) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ202.8, 202.6, 173.2. 172.0, 170.6, 170.5, 161.7, 139.7, 138.7, 138.7, 136.1, 136.1, 131.0, 130.9, 130.8, 127.3, 125.8, 125.8, 125.7, 125.1, 105.1, 105.0, 104.8, 85.3, 85.2, 83.5, 83.4, 58.9, 58.5, 58.0, 52.3, 52.2, 47.6, 34.7, 34.6, 33.0, 32.9, 24.6, 24.1, 23.9; ^{19}F NMR (376 MHz,d_6-DMSO) (proton decoupled) δ -226.68, -226.72, -230.50, -231.17, -232.60, -232.66; MS ES (+) 439.0 (M+H).

Example 22

5-Fluoro-4-oxo-3-[(2S)-2-(4-oxo-6-propylsulfanyl-4*H*-quinazolin-3-yl)-butyrylamino]-pentanoic acid

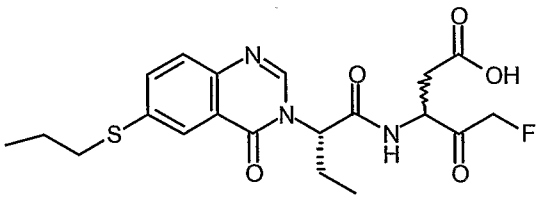


(S)-2-(4-oxo-6-propylsulfanyl-*4H*-quinazoline-3-yl)-butyric acid, *tert*-butyl ester



[0147] This compound was prepared from 5-chloro-2-nitrobenzoic acid methyl ester and propanethiol using procedures similar to those described in methods T-U and K-M. The sub-title compound was as a solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (3H, t), 1.10 (3H, t), 1.51 (9H, s), 1.75 (2H, m), 2.01 (1H, m), 2,31 (1H, m), 3.03 (2H, m), 5.30 (1H, m), 7.60-7.70 (2H, m), 8.05 (1H, s), 8.13 (1H, s) ppm; MS ES (+) 363.29 (M+H).

5-Fluoro-4-oxo-3-[(2S)-2-(4-oxo-6-propylsulfanyl-4*H*-quinazolin-3-yl)-butyrylamino]-pentanoic acid



[0148] This compound was prepared from (S)-2-(4-oxo-6-propylsulfanyl-*4H*-quinazoline-3-yl)-butyric acid (synthesized from the *tert*-butyl ester using TFA/DCM) using a procedure similar to that described in method F. The resulting alcohol was oxidized using tetra-n-propylammonium perruthenate(VII)/N-methylmorpholine-N-oxide (*Synthesis*, 639 (1994)) and the *tert*-butyl ester deprotected as described in method E. The title compound was obtained as a white solid (90% last step)(TFA salt).

IR (solid) 2963.9, 1786.2, 1739.4, 1666.8, 1195.2 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (3H, t), 1.08 (3H, t), 1.70-1.80 (2H, m), 2.02-2.09 (1H, m), 2.29-2.36 (1H, m), 2.72-2.92 (1H, m), 3.00-3.15 (1H, m), 3.03 (2H, t), 4.50-5.20 (3H, m), 5.40-5.60 (1H, m), 7.69-7.75 (2H, m), 8.06-8.07 (1H, m), 8.60-8.64 (1H, m) ppm; ^{19}F NMR (376 MHz,$CDCl_3$) (proton decoupled) -76.23, -231.5; MS ES(+) 436.3 (M+H).

Example 23

Enzyme Assays

[0149] The assays for caspase inhibition are based on the cleavage of a fluorogenic substrate by recombinant, purified human Caspases -1, -3, -7 or -8. The assays are run in essentially the same way as those reported by Garcia-Calvo et al., *J. Biol. Chem.* **273**, 32608-32613 (1998), using a substrate specific for each enzyme. The substrate for Caspase-1 is Acetyl-Tyr-Val-Ala-Asp-amino-4-methylcoumarin and the substrate for Caspases -3, -7 and -8 is Acetyl-Asp-Glu-Val-Asp-amino-4-methylcoumarin both as disclosed in Garcia-Calvo et al., *J. Biol. Chem.* **273**, 32608-32613 (1998).

[0150] The observed rate of enzyme inactivation at a particular inhibitor concentration, k_{obs}, is computed by direct fits of the data to the equation derived by Thornberry et al., *Biochemistry,* **33,** 3943-3939 (1994) using a nonlinear least-squares analysis computer program (PRISM 2.0; GraphPad software). To obtain the second order rate constant, k_{inact}, k_{obs} values are plotted against their respective inhibitor concentrations and k_{inact} values are subsequently calculated by computerized linear regression.

[0151] Table 2 shows inhibition of caspases-1, -3, and -8 activity for selected compounds of this invention as determined by the above method. Compounds with $k_{inact}(M^{-1}s^{-1})$ > 500000 are listed as "A". Compounds with $k_{inact}(M^{-1}s^{-1})$ between 100000 and 500000 are listed as "B". Compounds with $k_{inact}(M^{-1}s^{-1})$ < 100000 are listed as "C".

Table 2. Inhibition of Caspases-1, -3, and -8

Compound Number	k_{inact}		
	Caspase-1	Caspase-3	Caspase-8
1	A	A	B
2	A	A	A
3	B	B	C
4	B	B	C
5	A	A	B
6	B	A	C
7	C	B	C
8	B	B	C
9	B	B	C
10	A	A	C
11	A	A	C
12	A	A	A
13	B	B	A
14	A	A	B
15	B	B	A
16	A	A	A
17	A	A	A
18	B	B	C
19	A	A	C
20	A	A	C
21	A	A	A

Compound Number	k_{inact}		
	Caspase-1	Caspase-3	Caspase-8
22	A	A	A

Example 24

Inhibition of IL-1β secretion from Whole Blood

[0152] Human blood is freshly drawn from healthy donors and diluted 1:2 in PBS. To 500 µl of diluted blood 50ml of prediluted test compound in RPMI medium and 10 ml LPS (5ng/ml final concentration on the plate) are added (LPS, Serotype 0111:B4, Sigma L3012). After stimulation for 18 hours supernatants are collected and assayed for IL-1β levels using the appropriate ELISA kit (R&D systems).

[0153] Table 3 below shows inhibition of IL-1β secretion from human whole blood for selected compounds of this invention as determined by the above methods. Compounds with an IC_{50} >10µM are listed as "A". Compounds with an IC_{50} between 1µM and 10uM are listed as "B". Compounds with an IC_{50} <1µM are listed as "C".

Table 3. Inhibition of IL-1β secretion

Compound Number	IC_{50} (µm)
1	C
2	C
3	B
4	B
5	B
6	B
7	A
8	B

Compound Number	IC_{50} (μm)
9	A
10	B
11	A
12	B
13	B
14	C
15	B
16	B
17	B
18	B
19	B
20	A
21	B
22	C

Example 25

Hypoxia-induced apoptosis of rat cortical neurons

[0154] Cortical neurons are dissociated from Wistar rat embryos (E17) by a modification of the procedure of Rogers et al., *Brain Res. Bulletin*, **44**:131 (1998). Briefly, cerebral cortices are isolated aseptically from 15-20 Wistar rat embryos. A cell suspension is prepared by mincing the cerebral cortices and digesting them with papain. Cells are washed with ovomucoid enzyme inhibitor and DNaseI and plated onto Poly-D lysine coated plates in high glucose DMEM containing 10% heat-inactivated fetal calf serum, L-glutamine, penicillin and streptomycin. The yield of neurons is 10x7 per embryo and they are 80-90% viable as assessed by Trypan blue exclusion.

[0155] The neurons are cultured in complete medium at 37 °C in a normal atmosphere for 48 hours prior to the

hypoxia experiments. For hypoxia, the normal cell medium is replaced by oxygen-depleted serum-free medium. Cells are incubated in an atmosphere of 95% N_2/5% CO_2 for various lengths of time. Compounds are dissolved in DMSO at 100mM then diluted in medium and added to the culture from time=0. The level of apoptosis is measured using a Cell Death Detection ELISA kit (Roche) which detects DNA fragmentation. Plates are read at 405nm. Controls included cells cultured in aerobic conditions in serum-containing medium (+serum) and cells cultured in aerobic conditions in serum-deprived medium (-serum).

[0156] Table 4 shows the results of the activity of selected compounds of this invention in the Hypoxia-induced apoptosis of rat cortical neurons. Compounds with an IC_{50} >0.5μM are listed as "A". Compounds with an IC_{50} between 0.1μM and 0.5uM are listed as "B". Compounds with an IC_{50} <0.1μM are listed as "C".

Table 4. Activity in Hypoxia-induced Apoptosis Assay

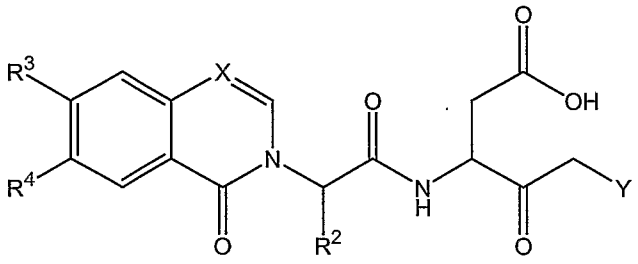
Compound Number	IC_{50} (μm)
1	B
2	A
3	B
4	A
5	B
6	B
7	A
8	B
9	B
10	C
11	B

Compound Number	IC_{50} (μm)
12	A
13	B
14	A
15	B
16	B
17	B
18	B
19	C
20	B
21	B
22	A

[0157] While we have described a number of embodiments of this invention, it is apparent that our basic examples may be altered to provide other embodiments that utilize the compounds and methods of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the appended claims rather than by the specific embodiments that have been represented by way of example above.

We claim:

1. A compound of formula **I**:



I

X is CH or N;

Y is halo, trifluorophenoxy, or tetrafluorophenoxy;

R^2 is C_{1-6} straight chained or branched alkyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3; and

R^4 is hydrogen, halo, OCF_3, SR, CN, CF_3, Ar, or T-Ar;

wherein:

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

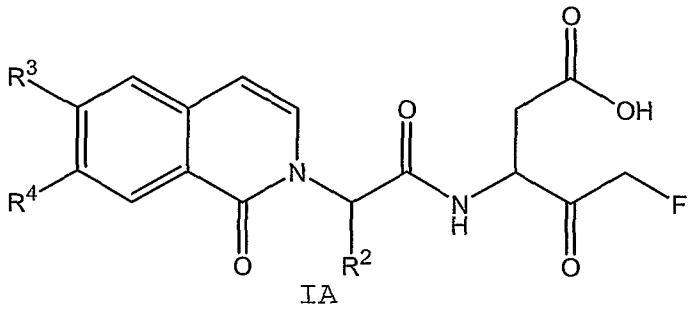
R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl);

provided that when Y is halo, then both, R^3 and R^4, are not simultaneously hydrogen.

2. The compound according to claim 1, wherein R^2 is ethyl, n-propyl, or isopropyl.

3. The compound according to claim 1 or claim 2, wherein Y is F, trifluorophenoxy, or tetrafluorophenoxy.

4. The compound according to claim 1, having formula IA:



IA

wherein:

R^2 is ethyl, n-propyl, or isopropyl; and

R^3 and R^4 are each independently hydrogen, halo, OCF_3, CN, CF_3 or Ar, provided that both, R^3 and R^4, are not simultaneously hydrogen.

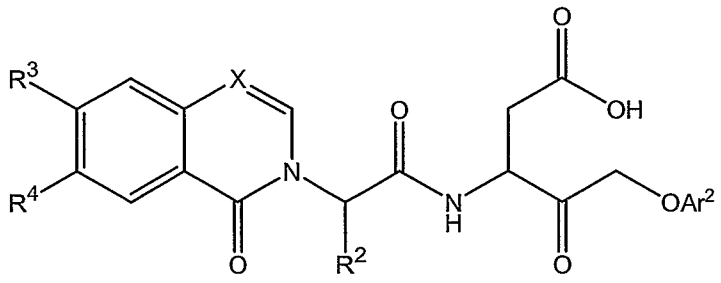
5. The compound according to claim 4, wherein R^2 is ethyl.

6. The compound according to claim 4 or claim 5, wherein R^3 is hydrogen.

7. The compound according to any one of claims 4-6, wherein R^3 is H, and R^4 is F, Cl, CN, Ar, or CF_3.

8. The compound according to any one of claims 4-7, wherein R^4 is Cl or CF_3.

9. The compound according to claim 1, having the formula IB:



IB

wherein:

X is CH or N;

R^2 is ethyl, n-propyl, or isopropyl;

R^3 and R^4 are each independently hydrogen, halo, OCF_3, CN, or CF_3; and

Ar^2 is trifluorophenyl or tetrafluorophenyl.

10. The compound according to claim 9, wherein Ar^2 is 2,3,5,6-tetrafluorophenyl.

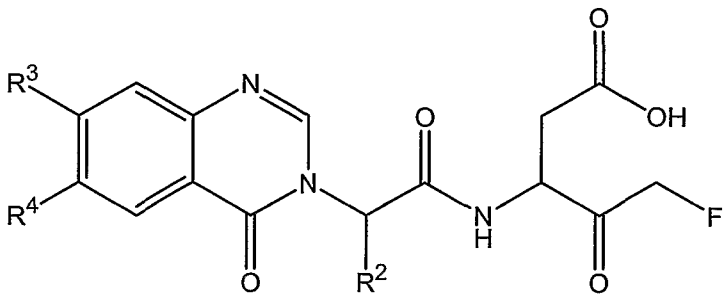
11. The compound according to claim 9 or claim 10, wherein R^2 is ethyl.

12. The compound according to any one of claims 9-11, wherein X is CH.

13. The compound according to any one of claims 9-12, wherein R^3 is H, and R^4 is F, Cl, or CF_3.

14. The compound according to claim 12 or claim 13, wherein R^4 is Cl or CF_3.

15. The compound according to claim 1, having formula IC:



IC

wherein:

R^2 is ethyl, n-propyl, or isopropyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3;

R^4 is halo, OCF_3, CN, CF_3, SR, or T-Ar;

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl).

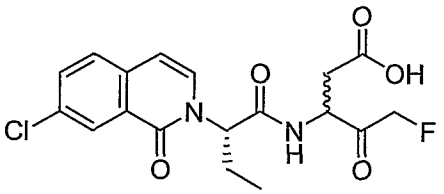
16. The compound according to claim 15, wherein R^2 is ethyl.

17. The compound according to claim 15 or claim 16, wherein R^3 is hydrogen.

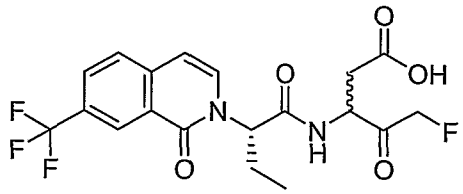
18. The compound according to any one of claims 15-17, wherein T is O.

19. The compound according to any one of claims 15-18, wherein R^4 is T-Ar.

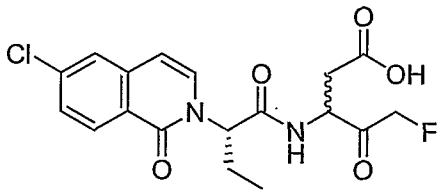
20. The compound of claim 1, selected from:



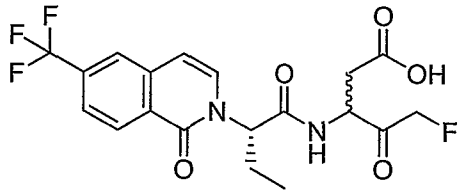
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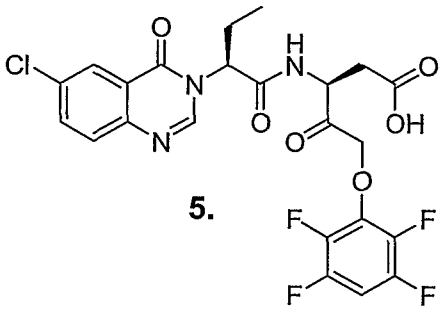
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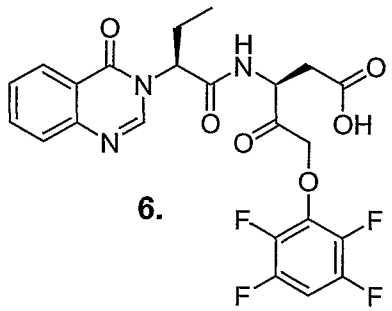
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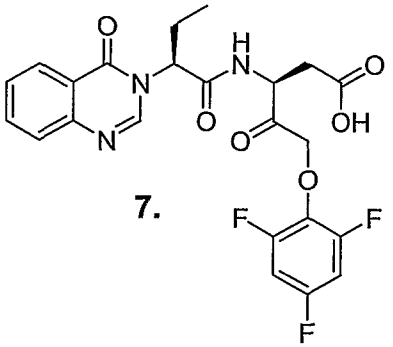
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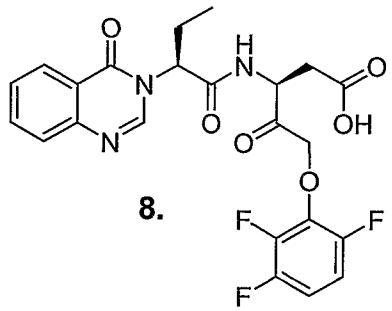
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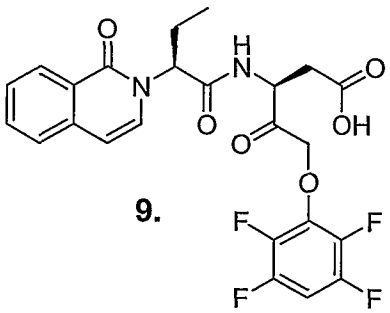
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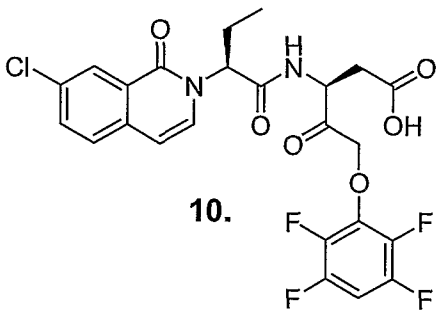
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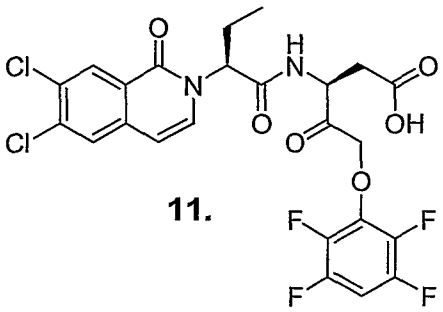
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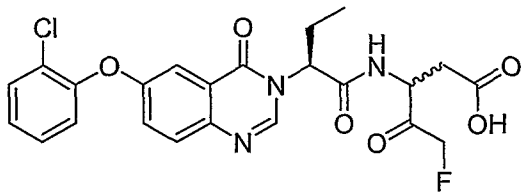
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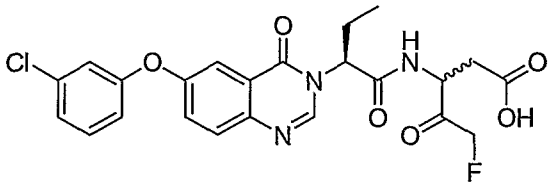
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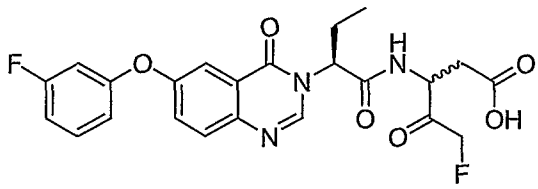
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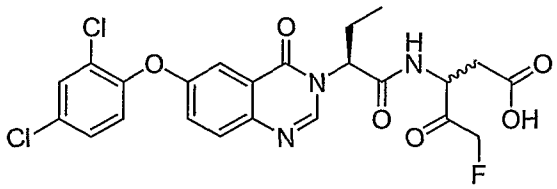
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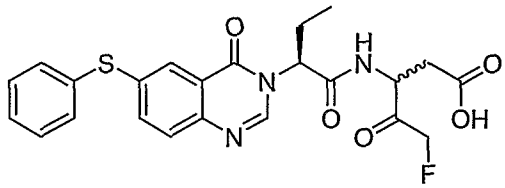
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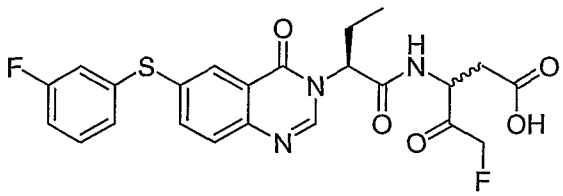
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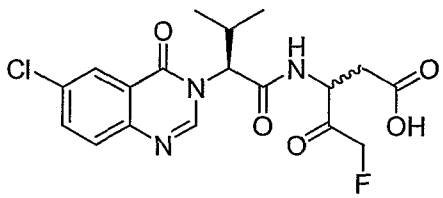
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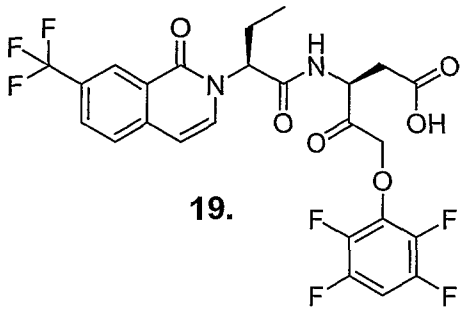
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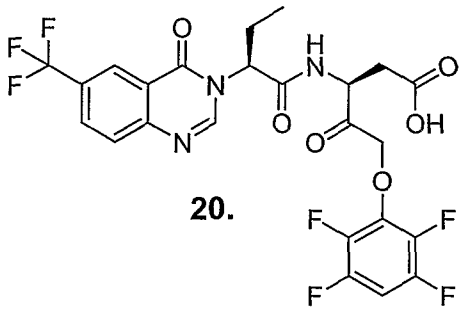
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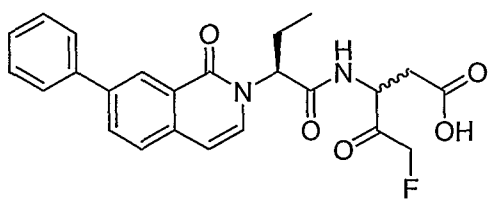
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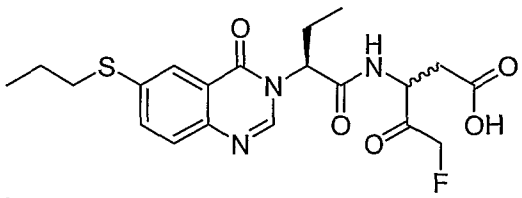


20.



21.

and



22.

21. A pharmaceutical composition comprising:

a) a compound according to any one of claims 1-20; and

b) a pharmaceutically acceptable carrier, adjuvant or vehicle.

22. A method for treating a disease in a patient, wherein said disease is selected from an IL-1 mediated disease, an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, retinal disorders, uveitis, inflammatory peritonitis, osteoarthritis, pancreatitis, asthma, adult respiratory distress syndrome, glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic thyroiditis, Grave's disease, autoimmune gastritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, myasthenia gravis, inflammatory bowel disease, Crohn's disease, psoriasis, atopic dermatitis, scarring, graft vs host disease, organ transplant rejection, organ apoptosis after burn injury, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, multiple myeloma-related bone disorder, acute myelogenous leukemia, chronic myelogenous leukemia, metastatic melanoma, Kaposi's sarcoma, multiple myeloma, haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart

disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, ulcerative colitis, traumatic brain injury, spinal cord injury, hepatitis-B, hepatitis-C, hepatitis-G, yellow fever, dengue fever, or Japanese encephalitis, various forms of liver disease, renal disease, polycystic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, meningitis, organ failure, treating complications associated with coronary artery bypass grafts, and an immunotherapy for the treatment of various forms of cancer;

said method comprising the step of administering to said patient a pharmaceutical composition according to claim 21.

23. The method according to claim 22, wherein the disease is an apoptosis mediated disease, an inflammatory disease, an autoimmune disease, a destructive bone disorder, a proliferative disorder, an infectious disease, a degenerative disease, a disease associated with cell death, an excess dietary alcohol intake disease, a viral mediated disease, inflammatory peritonitis, osteoarthritis, glomerulonephritis, rheumatoid arthritis, diabetes, autoimmune hemolytic anemia, autoimmune neutropenia, thrombocytopenia, chronic active hepatitis, scarring, graft vs host disease, organ transplant rejection, organ apoptosis after burn injury, osteoporosis, leukemias and related disorders, myelodysplastic syndrome, metastatic melanoma,

haemorrhagic shock, sepsis, septic shock, burns, Shigellosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, Kennedy's disease, prion disease, cerebral ischemia, epilepsy, myocardial ischemia, acute and chronic heart disease, myocardial infarction, congestive heart failure, atherosclerosis, coronary artery bypass graft, spinal muscular atrophy, amyotrophic lateral sclerosis, multiple sclerosis, HIV-related encephalitis, aging, alopecia, neurological damage due to stroke, traumatic brain injury, spinal chord injury, hepatitis-B, hepatitis-C, hepatitis-G, various forms of liver disease, renal disease, polycystic kidney disease, H. pylori-associated gastric and duodenal ulcer disease, HIV infection, tuberculosis, meningitis, treating complications associated with coronary artery bypass grafts, and an immunotherapy for the treatment of various forms of cancer.

24. The method according to claim 23, wherein said disease is complications associated with coronary artery bypass grafts.

25. A method for inhibiting a caspase-mediated function in a patient comprising the step of administering to said patient a pharmaceutical composition according to claim 21.

26. A method for decreasing IGIF or IFN-γ production in a patient comprising the step of administering to said patient a pharmaceutical composition according to claim 21.

27. A method of preserving cells, said method comprising the step of bathing the cells in a solution of the compound according to any one of claims 1-20 or a pharmaceutically acceptable derivative thereof.

28. The method according to claim 27, wherein said cells are in:

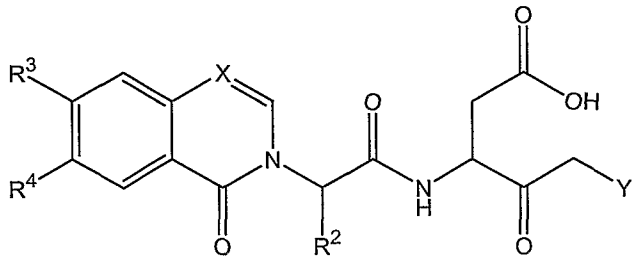
a) an organ intended for transplant; or

b) a blood product.

29. A method of treating cancer using immunotherapy, wherein said immunotherapy comprises as a component thereof a compound according to any one of claim 1-20.

30. The method according to any one of claims 22-29 wherein the composition, solution, or immunotherapy comprises an additional therapeutic agent.

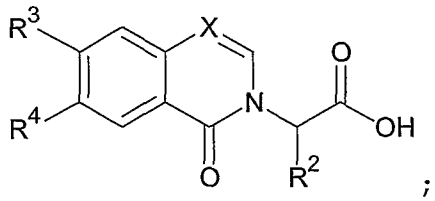
31. A method of preparing a compound of formula I,



I

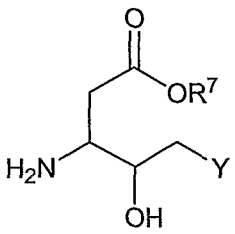
said method comprising:

reacting an acid or acid derivative of formula II,

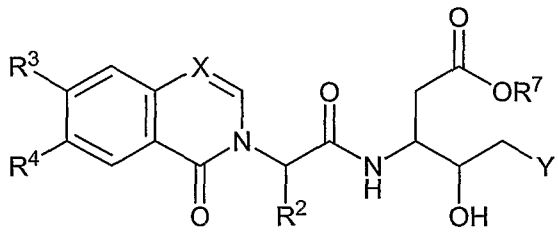


II

with an amino alcohol of formula B, to provide a compound of formula III,



B



III; and

converting intermediate III to compound I, wherein;

X is CH or N;

Y is halo, trifluorophenoxy, or tetrafluorophenoxy;

R^2 is C_{1-6} straight chained or branched alkyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3; and

R^4 is hydrogen, halo, OCF_3, SR, CN, CF_3, Ar, or T-Ar;

wherein:

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

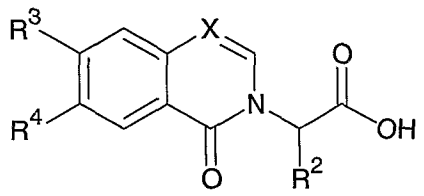
Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6;

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl); and

R^7 is a suitable protecting group;

provided that when Y is halo, then both, R^3 and R^4, are not simultaneously hydrogen.

32. A compound of formula IIA:



IIA

wherein;

X is CH or N;

R^2 is C_{1-6} straight chained or branched alkyl;

R^3 is hydrogen, halo, OCF_3, CN, or CF_3; and

R^4 is hydrogen, halo, OCF_3, SR, CN, CF_3, Ar, or T-Ar;

wherein:

T is O or S;

R is a C_{1-6} straight chained or branched alkyl;

Ar is a phenyl ring optionally substituted with 1-3 groups selected from halo, CH_3, CF_3, CN, OMe, OCF_3, and NR^5R^6; and

R^5 and R^6 each is independently H or C_{1-6} straight chained or branched alkyl, or R^5 and R^6, taken together, form a 5-7 membered ring optionally containing up to 3 heteroatoms selected from O, S, NH, and N(C_{1-6}-straight chained or branched alkyl).

33. The compound according to claim 32 wherein R^2 is ethyl or isopropyl.