PYRIMIDINE DERIVATIVES AND THEIR USE IN THE TREATMENT OF RESPIRATORY DISEASES SUCH AS COPD

Field of the Invention

This invention relates to heterocyclic compounds, which are pyrimidine derivatives having human neutrophil elastase inhibitory properties, and their use in therapy.

Background to the invention

Human neutrophil elastase (HNE) is a 32 kDa serine proteinase found in the azurophilic granules of neutrophils. It has a role in the degradation of a wide range of extracellular matrix proteins, including fibronectin, laminin, proteoglycans, Type III and Type IV collagens as well as elastin (Bieth, G. In *Regulation of Matrix accumulation*, Mecham, R. P. (Eds), Academic Press, NY, USA 1986, 217-306). HNE has long been considered to play an important role in homeostasis through repair and disposal of damaged tissues via degradation of the tissue structural proteins. It is also relevant in the defence against bacterial invasion by means of degradation of the bacterial body. In addition to its effects on matrix tissues, HNE has been implicated in the upregulation of IL-8 gene expression and also induces IL-8 release from the epithelial cells of the lung. In animal models of Chronic Obstructive Pulmonary Disease induced by tobacco smoke exposure both small molecule inhibitors and protein inhibitors of HNE inhibit the inflammatory response and the development of emphysema (Wright, J. L. et al. *Am. J. Respir. Crit. Care Med.* 2002, *166*, 954-960; Churg, A. et al. *Am. J. Respir. Crit. Care Med.* 2003, *168*, 199-207). Thus, HNE may play a role both in matrix destruction and in amplifying inflammatory responses in chronic respiratory diseases where neutrophil influx is a characteristic feature. Indeed, HNE is believed to play a role in several pulmonary diseases, including chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), pulmonary emphysema, pneumonia and lung fibrosis. It is also implicated in several cardiovascular diseases in which tissue remodelling is involved, for example, in heart failure and the generation of ischaemic tissue injury following acute myocardial infarction.

COPD is an umbrella term encompassing three different pathological conditions, all of which contribute to limitation of airflow: chronic bronchitis, emphysema and small-airway disease. Generally all three will exist to varying extents in patients presenting with COPD, and all three may be due to neutrophil-mediated inflammation, as supported by the increased number of neutrophils observed in bronchoalveolar leakage (BAL) fluids of COPD patients (Thompson, A. B.; Daughton, D.; et al. *Am.*

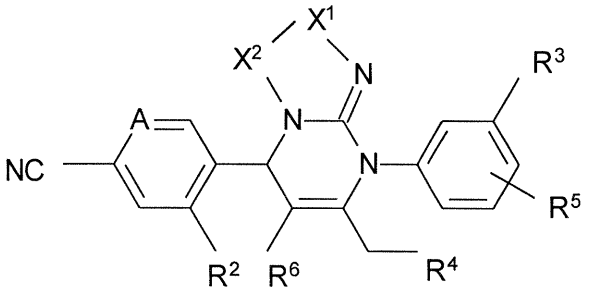
Rev. Respir. Dis. 1989, *140*, 1527-1537). The major pathogenic determinant in COPD has long been considered to be the protease-anti-protease balance (also known as the 'elastase:anti-elastase hypothesis'), in which an imbalance of HNE and endogenous antiproteases such as α 1-antitrypsin (α_1-AT), secretory leukocyte protease inhibitor (SLPI) and pre-elafin leads to the various inflammatory disorders of COPD. Individuals that have a genetic deficiency of the protease inhibitor α 1-antitrypsin develop emphysema that increases in severity over time (Laurrell, C. B.; Erikkson, S *Scand. J. Clin. Invest.* 1963 *15*, 132-140). An excess of HNE is therefore destructive, leading to the breakdown of pulmonary morphology with loss of elasticity and destruction of alveolar attachments of airways in the lung (emphysema) whilst simultaneously increasing microvascular permeability and mucus hypersecretion (chronic bronchitis).

Brief description of the invention

This invention provides novel compounds which are inhibitors of HNE, and are useful in the treatment of diseases or conditions in which HNE activity plays a part.

Detailed Description of the Invention

In one embodiment, the invention provides a compound of formula (I):



(I)

wherein

A is C-R^1;

$\mathbf{R^1}$ and $\mathbf{R^2}$ are hydrogen;

$\mathbf{R^3}$ is 3-trifluoromethyl, 3-chloro or 3-bromo;

$\mathbf{R^4}$ is hydrogen;

$\mathbf{R^5}$ is hydrogen;

$\mathbf{R^6}$ is -$CO_2\mathbf{R^{11}}$;

$-X^1-X^2-$ is $-NR^{19}-CO-$;

R^{11} represents a radical of formula $-[Alk^1]_p-[Q]_t-[Alk^2]_q-Z$ wherein

p and **q** are independently 0 or 1;

t is 0;

Alk^1 and **Alk**^{2} each independently represent a C_1-C_6 alkylene radical;

Q represents a divalent mono- or bicyclic carbocyclic or heterocyclic radical having 3-9 ring members;

Z is

(i) $-N(R^A)(R^B)$ wherein R^A and R^B are independently hydrogen, or a C_1-C_6-alkyl; or

(ii) $-N^+(R^A)(R^B)(R^C)$ wherein
R^A, R^B and R^C are independently a C_1-C_6-alkyl;

R^{19} is selected from hydrogen, and (C_1-C_6)alkyl.

Compounds of formula (I) above thereof may be prepared in the form of salts, particularly pharmaceutically acceptable salts, N-oxides, hydrates and solvates thereof. Any claim to a compound herein, or reference to "compounds of the invention", "compounds with which the invention is concerned", and "compounds of formula (I)" includes such compounds whether or not in salt, N-oxide, hydrate or solvate form.

Compounds of the invention may be used in the treatment or prevention of diseases in which HNE is implicated, for example chronic obstructive pulmonary disease (COPD), chronic bronchitis, lung fibrosis, pneumonia, acute respiratory distress syndrome (ARDS), pulmonary emphysema, smoking-induced emphysema and cystic fibrosis.

Hence other aspects of the invention are (i) a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier or excipient; and

(ii) the use of a compound of the invention for the manufacture of a medicament for the treatment or prevention of a disease or condition in which HNE is implicated.

Terminology

As used herein, the term "C_a-C_b-alkyl" wherein a and b are integers refers to a straight or branched chain alkyl radical having from a to b carbon atoms. Thus when a is 1 and b is 6, for example, the term includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl and n-hexyl.

As used herein the term "C_a-C_b-alkenyl" wherein a and b are integers refers to a straight or branched chain alkenyl moiety having from a to b carbon atoms having at least one double bond of either E or Z stereochemistry where applicable. Thus when a is 2 and b is 6, for example, the term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein the term "C_a-C_b-alkynyl" wherein a and b are integers refers to straight chain or branched chain hydrocarbon groups having from a to b carbon atoms and having in addition one triple bond. Thus when a is 1 and b is 6, for example, the term includes for example, ethynyl (-C≡CH), 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl and 5-hexynyl.

As used herein the term "divalent C_a-C_b-alkylene radical" wherein a and b are integers refers to a saturated hydrocarbon chain having from a to b carbon atoms and two unsatisfied valences.

As used herein the term "divalent C_a-C_b-alkenylene radical" wherein a and b are integers refers to a divalent hydrocarbon chain having from a to b carbon atoms, and at least one double bond.

As used herein the unqualified term "carbocyclic" refers to a mono-, bi- or tricyclic radical or bridged monocyclic or bicyclic radical having up to 16 ring atoms, all of which are carbon, and includes aryl and cycloalkyl. A bridged carbocyclic radical has a monocyclic or bicyclic ring with two ring atoms joined by an alkylene bridge, such as radicals of bicyclo[2.2.2]octane, bicyclo[3.1.1]heptane and adamantane.

As used herein the unqualified term "cycloalkyl" refers to a monocyclic saturated carbocyclic radical having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, bicyclo[2.2.2]octanyl and adamantanyl.

As used herein the unqualified term "aryl" refers to a mono-, bi- or tri-cyclic carbocyclic aromatic radical, and includes radicals having two monocyclic carbocyclic aromatic rings which are directly linked by a covalent bond. Illustrative of such radicals are phenyl, biphenyl and napthyl.

As used herein the unqualified term "heteroaryl" refers to a mono-, bi- or tri-cyclic aromatic radical containing one or more heteroatoms selected from S, N and O, and includes radicals having two such monocyclic rings, or one such monocyclic ring and one monocyclic aryl ring, which are directly linked by a covalent bond. Illustrative of such radicals are thienyl, benzthienyl, furyl, benzfuryl, pyrrolyl, imidazolyl, benzimidazolyl, thiazolyl, benzthiazolyl, isothiazolyl, benzisothiazolyl, pyrazolyl, oxazolyl, benzoxazolyl, isoxazolyl, benzisoxazolyl, isothiazolyl, triazolyl, benztriazolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolyl and indazolyl. Where a heteroaryl ring contains an sp2 nitrogen such as in the case of pyridine or imidazole, that nitrogen may be a quaternary nitrogen as in pyridinium or imidazolium.

As used herein the unqualified term "heterocyclyl" or "heterocyclic" or "heterocycloalkyl" includes "heteroaryl" as defined above, and in its non-aromatic meaning relates to a mono-, bi- or tri-cyclic or bridged monocyclic or bicyclic non-aromatic radical containing one or more heteroatoms selected from S, N and O, and to groups consisting of a monocyclic non-aromatic radical containing one or more such heteroatoms which is covalently linked to another such radical or to a monocyclic carbocyclic radical. A bridged heterocyclic radical has a monocyclic or bicyclic ring containing at least one S, N or O ring atom with two ring atoms, such as two ring carbons, or a ring nitrogen and a ring carbon, joined by an alkylene bridge, such as radicals of 1-aza-bicyclo[2.2.2]octane. Where a ring nitrogen is bridged in this way, it may be further substituted as a quaternary nitrogen centre. Illustrative of such radicals are pyrrolyl, furanyl, thienyl, piperidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, morpholinyl, benzfuranyl, pyranyl, isoxazolyl, benzimidazolyl, methylenedioxyphenyl, ethylenedioxyphenyl, maleimido, succinimido and 1-aza-bicyclo[2.2.2]octanyl or 1 methyl-1-aza-bicyclo[2.2.2]octanyl groups.

Unless otherwise specified in the context in which it occurs, the term "substituted" as applied to any moiety herein means substituted with up to four compatible substituents, each of which independently may be, for example, C_1-C_6-alkyl, cycloalkyl, C_1-C_6-alkoxy, hydroxy, hydroxyl-C_1-C_6-alkyl, mercapto, mercapto-C_1-C_6-alkyl, C_1-C_6-alkylthio, phenyl, monocyclic heteroaryl having 5 or 6 ring atoms, halo (including fluoro, bromo and chloro), trifluoromethyl, trifluoromethoxy, nitro, nitrile (-CN), oxo, -COOH, -$COOR^G$, -COR^G, -SO_2R^G, -$CONH_2$, -SO_2NH_2, -$CONHR^G$,

$-SO_2NHR^G$, $-CONR^GR^H$, $-SO_2NR^GR^H$, $-NH_2$, $-NHR^G$, $-NR^GR^H$, $-OCONH_2$, $-OCONHR^G$, $-OCONR^GR^H$, $-NHCOR^G$, $-NHCOOR^G$, $-NR^HCOOR^G$, $-NHSO_2OR^G$, $-NR^HSO_2OH$, $-NR^HSO_2OR^G$,$-NHCONH_2$, $-NR^GCONH_2$, $-NHCONHR^H$, $-NR^GCONHR^H$, $-NHCONR^GR^H$, or $-NR^GCONR^GR^H$ wherein R^G and R^H are independently a C_1-C_6-alkyl, C_3-C_6-cycloalkyl , phenyl or monocyclic heteroaryl having 5 or 6 ring atoms, or R^G and R^H when attached to the same nitrogen atom form a cyclic amino ring, such as piperidinyl, morpholinyl or piperazinyl. An "optional substituent" may be one of the foregoing substituent groups.

As used herein the term "salt" includes base addition and acid addition salts Compounds of the invention which are acidic can form salts, including pharmaceutically acceptable salts, with bases such as alkali metal hydroxides, e.g. sodium and potassium hydroxides; alkaline earth metal hydroxides e.g. calcium, barium and magnesium hydroxides; with organic bases e.g. N-methyl-D-glucamine, choline tris(hydroxymethyl)amino-methane, L-arginine, L-lysine, N-ethyl piperidine, and dibenzylamine. Those compounds (I) which are basic can form salts, including pharmaceutically acceptable salts with inorganic acids, e.g. with hydrohalic acids such as hydrochloric or hydrobromic acids, sulphuric acid, and nitric acid or phosphoric acid, and with organic acids e.g. with acetic, tartaric, succinic, fumaric, maleic, malic, salicylic, citric, methanesulphonic, p-toluenesulphonic, benzoic, benzenesunfonic, glutamic, lactic, and mandelic acids. Those compounds (I) which have a quaternary nitrogen can also form quaternary salts with a pharmaceutically acceptable counter-ion such as chloride, bromide, acetate, formate, p-toluenesulfonate, succinate, hemi-succinate, naphthalene-bis sulfonate, methanesulfonate, and xinafoate.

Compounds of the invention which contain one or more actual or potential chiral centres, because of the presence of asymmetric carbon atoms, can exist as a number of diastereoisomers with R or S stereochemistry at each chiral centre. The invention includes all such diastereoisomers and mixtures thereof.

In the compounds of the invention of formula (I), in any compatible combination:

The ring containing A is a phenyl ring.

R^1 and R^2 are hydrogen.

In one type of compound of the invention R^5 is hydrogen and R^3 is 3-trifluoromethyl, 3-

chloro or 3-bromo.

R^{6} is -CO_2**R**^{11}, wherein **R**^{11} represents a radical of formula -[Alk^1]$_p$-[Q]$_t$-[Alk^2]$_q$-Z wherein

p and **q** are independently 0 or 1;

t is 0;

Alk^{1} and **Alk**^{2} each independently represent a C_1-C_6 alkylene radical such as –CH_2-, –CH_2CH_2- or –$CH_2CH_2CH_2$-;

Q represents a divalent mono- or bicyclic carbocyclic or heterocyclic radical having 3-9 ring members, such as a 1,3-cyclopentylene, 1,4-cyclohexylene, 1-4-phenylene, 2,5-pyridinylene, 1,4-piperidinylene, or 1,4-piperazinylene radical;

Z is

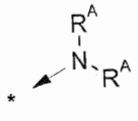
(i) -N(R^A)(R^B) wherein R^A and R^B are independently hydrogen, or a C_1-C_6-alkyl group such as methyl, ethyl or n- or isopropyl; or

(ii) -N^+(R^A)(R^B)(R^C) wherein
R^A, R^B and R^C are independently a C_1-C_6-alkyl group such as methyl, ethyl or n- or isopropyl.

More complex R^6 substituents include those represented by -CO_2**R**^{11}, wherein R^{11} has the formula -[Alk^1]$_p$-[Q]$_t$-[Alk^2]$_q$-Z wherein -[Alk^1]$_p$-[Q]$_t$-[Alk^2]$_q$- is structure (IV) wherein V^1 is 0, 1, 2, 3 or 4, and Z is structure (VI) wherein R^A is as defined in relation to formula (I).



(IV)



(VI)

Amine and pyridine nitrogen atoms, where present in group (VI), may be quaternised with an optionally substituted C_1-C_6-alkyl.

In one type of compound of the invention, R^6 has the formula $-CO_2R^{11}$ wherein R^{11}, represent a radical of formula -Alk^2-Z, wherein Alk^2 and Z are as defined and discussed above. For example in this type of compound, Alk^2 may be $–CH_2$-, $–CH_2CH_2$- or $–CH_2CH_2CH_2$- and Z may be $-N(R^A)(R^B)$ or $-N^+(R^A)(R^B)(R^C)$ wherein R^A, R^B and R^C are independently a C_1-C_6-alkyl group such as methyl, ethyl or n- or isopropyl. Thus, R^6 may be $–CO_2CH_2CH_2N(CH_3)_2$ or $–CO_2CH_2CH_2N^+(CH_3)_3$

-X^1-X^2- is -N**R^{19}**-CO-, wherein R19 is as defined above in relation to formula (I). Thus **R^{19}** may be, for example hydrogen, methyl, ethyl, n- or iso-propyl.

Examples of specific groups R^1-R^6 include those present in the compounds of the Examples herein.

The therapeutic utility of the present compounds is pertinent to any disease that is known to be at least partially mediated by the action of human neutrophil elastase. For example, the present compounds may be beneficial in the treatment of chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), pulmonary emphysema, pneumonia and lung fibrosis.

The present invention is also concerned with pharmaceutical formulations comprising, as an active ingredient, a compound of the invention. Other compounds may be combined with compounds of this invention for the prevention and treatment of inflammatory diseases of the lung. Thus the present invention is also concerned with pharmaceutical compositions for preventing and treating inflammatory diseases of the lung comprising a therapeutically effective amount of a compound of the invention and one or more other therapeutic agents.

Suitable therapeutic agents for a combination therapy with compounds of the invention include: (1) a corticosteroid, for example fluticasone or budesonide; (2) a β2-adrenoreceptor agonist, for example salmeterol or formeterol; (3) a leukotriene modulator, for example montelukast or pranlukast; (4) anticholinergic agents, for example selective muscarinic-3 (M3) receptor antagonists such as tiotropium bromide; (5) phosphodiesterase-IV (PDE-IV) inhibitors, for example roflumilast or cilomilast; (6) an antitussive agent, such as codeine or dextramorphan; and (7) a non-steroidal anti-

inflammatory agent (NSAID), for example ibuprofen or ketoprofen.

The weight ratio of the first and second active ingredients may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used.

The magnitude of prophylactic or therapeutic dose of a compound of the invention will, of course, vary with the nature of the severity of the condition to be treated and with the particular compound and its route of administration, and will generally be detrmined by clinical trial as required in the pharmaceutical art. It will also vary according to the age, weight and response of the individual patient. In general, the daily dose range will lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be necessary to use dosages outside these limits in some cases.

Another aspect of the present invention provides pharmaceutical compositions which comprise a compound of the invention and a pharmaceutically acceptable carrier. The term "composition", as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of the invention, additional active ingredient(s), and pharmaceutically acceptable excipients.

The pharmaceutical compositions of the present invention comprise a compound of the invention as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

Any suitable route of administration may be employed for providing a mammal, especially a human, with an effective dosage of a compound of the present invention. In therapeutic use, the active compound may be administered by any convenient, suitable or effective route. Suitable routes of administration are known to those skilled in the art, and include oral, intravenous, rectal, parenteral, topical, ocular, nasal, buccal and pulmonary (by inhalation).

Compositions suitable for administration by inhalation are known, and may include

carriers and/or diluents that are known for use in such compositions. The composition may contain 0.01-99% by weight of active compound. Preferably, a unit dose comprises the active compound in an amount of 1µg to 10 mg.

The most suitable dosage level may be determined by any suitable method known to one skilled in the art. It will be understood, however, that the specific amount for any particular patient will depend upon a variety of factors, including the activity of the specific compound that is used, the age, body weight, diet, general health and sex of the patient, time of administration, the route of administration, the rate of excretion, the use of any other drugs, and the severity of the disease undergoing treatment.

For delivery by inhalation, the active compound is preferably in the form of microparticles. They may be prepared by a variety of techniques, including spray-drying, freeze-drying and micronisation.

By way of example, a composition of the invention may be prepared as a suspension for delivery from a nebuliser or as an aerosol in a liquid propellant, for example for use in a pressurised metered dose inhaler (PMDI). Propellants suitable for use in a PMDI are known to the skilled person, and include CFC-12, HFA-134a, HFA-227, HCFC-22 (CCl2F2) and HFA-152 (CH4F2 and isobutane).

In a preferred embodiment of the invention, a composition of the invention is in dry powder form, for delivery using a dry powder inhaler (DPI). Many types of DPI are known.

Microparticles for delivery by administration may be formulated with excipients that aid delivery and release. For example, in a dry powder formulation, microparticles may be formulated with large carrier particles that aid flow from the DPI into the lung. Suitable carrier particles are known, and include lactose particles; they may have a mass median aerodynamic diameter of greater than 90 µm.

In the case of an aerosol-based formulation, a preferred composition is:

Compound of the invention	24 mg / canister
Lecithin, NF Liq. Conc.	1.2 mg / canister
Trichlorofluoromethane, NF	4.025 g / canister
Dichlorodifluoromethane, NF	12.15 g / canister.

Compounds of the invention may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which present compounds are useful. Such other drugs may be administered, by a route and in an amount commonly used therefore, contemporaneously or sequentially with a compound of the invention. When a compound of the invention is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of the invention is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a compound of the invention.

The agents of the invention may be administered in inhaled form. Aerosol generation can be carried out using, for example, pressure-driven jet atomizers or ultrasonic atomizers, preferably using propellant-driven metered aerosols or propellant-free administration of micronized active compounds from, for example, inhalation capsules or other "dry powder" delivery systems.

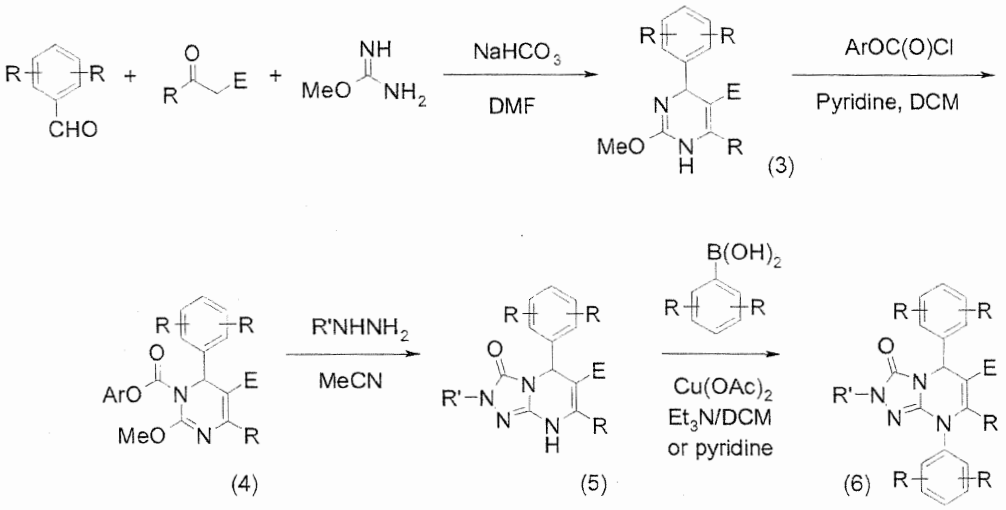
The active compounds may be dosed as described depending on the inhaler system used. In addition to the active compounds, the administration forms may additionally contain excipients, such as, for example, propellants (e.g. Frigen in the case of metered aerosols), surface-active substances, emulsifiers, stabilizers, preservatives, flavorings, fillers (e.g. lactose in the case of powder inhalers) or, if appropriate, further active compounds.

For the purposes of inhalation, a large number of systems are available with which aerosols of optimum particle size can be generated and administered, using an inhalation technique which is appropriate for the patient. In addition to the use of adaptors (spacers, expanders) and pear-shaped containers (e.g. Nebulator®, Volumatic®), and automatic devices emitting a puffer spray (Autohaler®), for metered aerosols, in particular in the case of powder inhalers, a number of technical solutions are available (e.g. Diskhaler®, Rotadisk®, Turbohaler® or the inhalers for example as described EP-A-0505321).

Methods of Synthesis

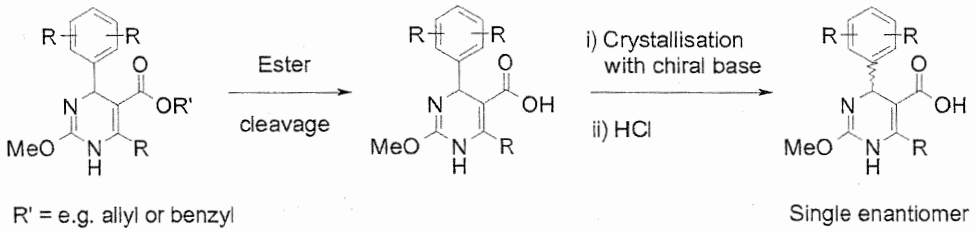
Compounds wherein X^1-X^2 is $-NR^{19}$-CO- can be prepared according to Schemes 5-10. Group R^{11} is a group of formula $-[Y]_m$-$[Alk^1]_p$-$[Q]_t$-$[Alk^2]_q$-Z or a group which can be later converted into such. For compounds wherein X^1-X^2 is $-NR^{19}$-CO-, the group may be incorporated for example, but not exclusively, according to Scheme 11.

Other examples of the invention (6) can be prepared according to Scheme 5. *O*-Methylurea, an aldehyde and a ketone with a beta electron-withdrawing group E (e.g. an ester, amide or ketone) may be reacted in the presence of a base, such as sodium hydrogen carbonate, to form an intermediate (3). Reaction with an activated chloroformate, such as 4-nitrophenylchloroformate or pentafluorophenylchloroformate, produces (4). Ring closure can then be achieved using a substituted hydrazine to give (5). Arylation of (5) can be effected using an arylboronic acid, such as 3-(trifluoromethyl)phenylboronic acid, under copper catalysis and in the presence of a base e.g. pyridine or triethylamine. The reaction can be carried out with or without solvent. The group R' may be removed under suitable conditions and when R' = H, the nitrogen atom may be alkylated or acylated using standard chemistry. All of the reactions may be performed in various solvents that must be compatible with the reagents used, and may be carried out at various suitable temperatures, typically 0-80°C depending on the solvent used.

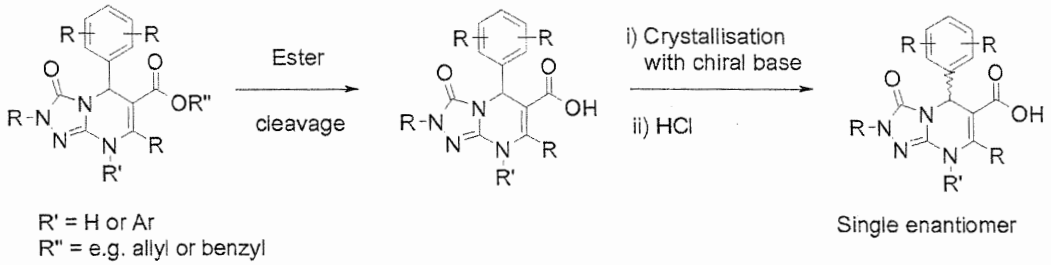


Scheme 5

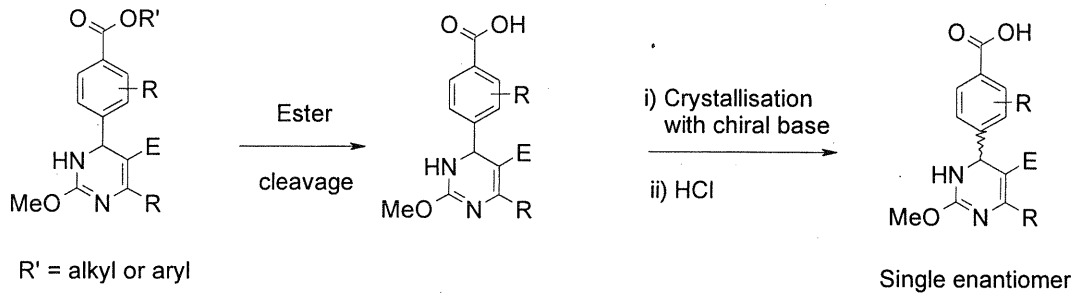
The enantiomers of racemic mixture (3), (5) or (6) may be separated by chiral HPLC. Schemes 6 to 9 show how the incorporation of a carboxylic acid group into a chiral intermediate may allow resolution by crystallisation of a chiral salt.



Scheme 6



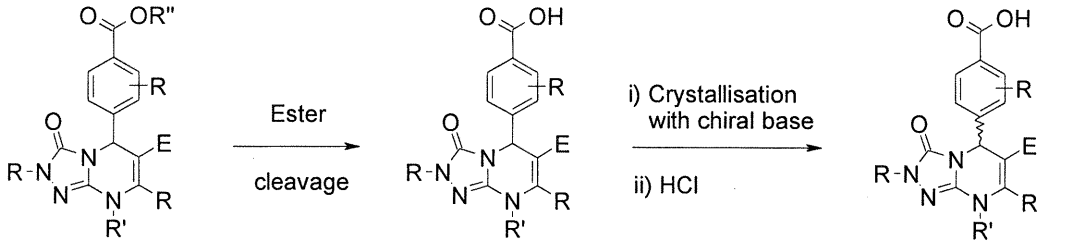
Scheme 7



R' = alkyl or aryl

Single enantiomer

Scheme 8

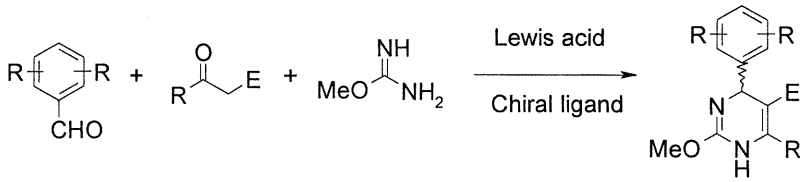


R' = H or Ar
R'' = alkyl or aryl

Single enantiomer

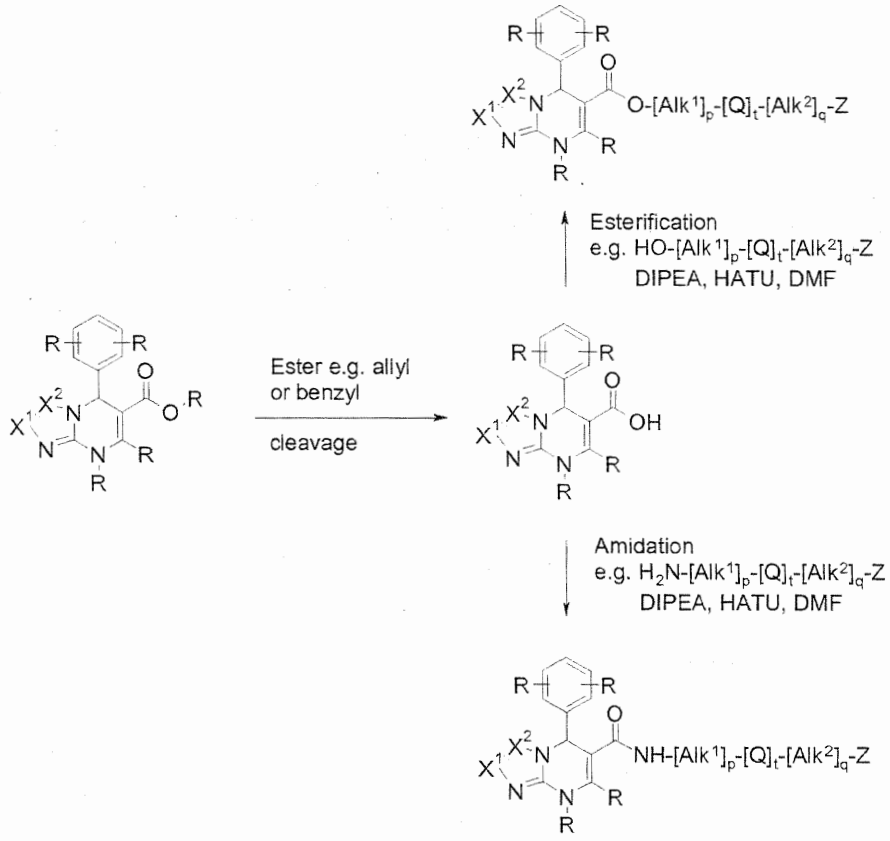
Scheme 9

The three component reaction of Scheme 5 may also be carried out in a stereospecific manner providing exclusively, or an enantiomeric excess of, the more active enantiomer (J. Am. Chem. Soc., 2005, 127, 16386-16387).



Scheme 10

In Schemes 5, 8, 9 and 10, group E may be modified at various stages using standard chemical methods. Scheme 11 describes how, when group E is an ester, this functional group may be transformed using chemistries available to those skilled in the art.



Scheme 11

General Experimental Details

Reactions were not carried out under an inert atmosphere unless specified. Where separation was carried out using a RediSep® Si cartridge, an automated chromatography system was used (CombiFlash® companion) together with a pre-packed polypropylene (RediSep®) column containing silica with average particle size 35-70 μm (230-400 mesh). 'Isolute ® PE-AX cartridge' refers to a pre-packed polypropylene column containing a silica-based sorbent with a chemically bonded quaternary ammonium functional group. All solvents and commercial reagents were used as received.

Preparative HPLC Conditions

HPLC System 1

C18-reverse-phase end-capped column (250 × 21.2 mm Gemini column with 5 μm particle size), eluting with a gradient of A: water; B: acetonitrile (0.1% formic acid added) with a flow rate typically 18 ml/min and gradient of 1 %/min increasing in B.

UV detection at 254 nm.

Analytical LC-MS Conditions

LC-MS Method 1

Waters Platform LC with a C18-reverse-phase column (30 × 4.6 mm Phenomenex Luna 3 µm particle size), elution with A: water + 0.1% formic acid; B: methanol + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B
0.00	2.0	95	5
0.50	2.0	95	5
4.50	2.0	5	95
5.50	2.0	5	95
6.00	2.0	95	5

Detection - MS, ELS, UV

MS ionisation method - Electrospray (positive and negative ion)

LC-MS Method 2

Waters Micromass ZQ2000 with a C18-reverse-phase column (100 × 2.1 mm Acquity BEH with 1.7 µm particle size) maintained at 40°C, elution with A: water + 0.1% formic acid; B: acetonitrile + 0.1 % formic acid. Gradient:

Gradient – Time	flow ml/min	%A	%B
0.00	0.4	95	5
0.40	0.4	95	5
6.00	0.4	5	95
6.80	0.4	5	95
7.00	0.4	95	5
8.00	0.4	95	5

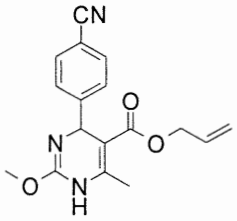
Detection - MS, UV PDA

MS ionisation method - Electrospray (positive/negative ion)

Abbreviations used in the experimental section:

DCM	dichloromethane
DIPEA	Di-isopropylethylamine
DMF	*N*,*N*-dimethylformamide
HATU	*O*-(7-Azabenzotriazole-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluoro-phosphate
HPLC	high performance liquid chromatography
IPA	Isopropyl alcohol
RT	room temperature
Rt	retention time

Intermediate 4



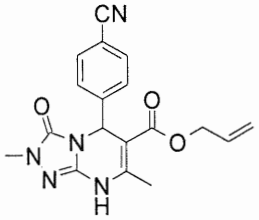
4-(4-Cyanophenyl)-2-methoxy-6-methyl-1,4-dihydro-pyrimidine-5-carboxylic acid allyl ester

4-Cyanobenzaldehyde (13.1 g, 100 mmol) was dissolved in DMF (200 ml) and sodium bicarbonate (33.4 g, 400 mmol) was added, followed by *O*-methylisourea hemisulphate (14.8 g, 120 mmol) and allyl acetoacetate (15.06 g, 110 mmol). The mixture was heated under nitrogen at 70°C overnight. After allowing to cool to RT, the mixture was filtered and the filtrate was evaporated. The resulting orange oil was partitioned between ethyl acetate and water. The organic layer was separated, washed with brine, dried (Na_2SO_4) and evaporated. The crude product was purified by flash silica chromatography eluting with 0-30% ethyl acetate in DCM to give pure Intermediate 4 as a yellow foam.

Yield: 16.8 g (53%)

LC-MS (Method 1): Rt = 2.18 min, m/z = 312 $[M+H]^+$

Intermediate 5

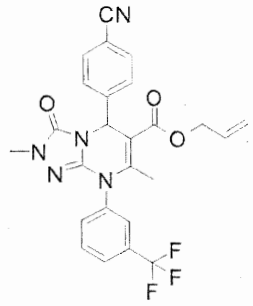


5-(4-Cyanophenyl)-2,7-dimethyl-3-oxo-2,3,5,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carboxylic acid allyl ester

Intermediate 4 (2.15 g, 6.91 mmol) and pyridine (4.5 ml) were dissolved in DCM (20 ml) and the solution was cooled in an ice bath. 4-Nitrophenyl chloroformate (1.38 g, 6.91 mmol) was added and the reaction was stirred whilst cooling was maintained.

After 2 hours methyl hydrazine (550 µl, 10.4 mmol) was added and, after 10 minutes, the reaction was diluted with DCM, washed with water, dried (Na_2SO_4) and evaporated. Traces of pyridine were removed as an azeotrope with toluene. The product was purified on a RediSep® Si column (120 g) eluting with 40-80% ethyl acetate in cyclohexane. Intermediate 5 was obtained as pale yellow solid.
Yield: 1.22 g (50%)
LC-MS (Method 1): Rt = 2.79 min, m/z = 352 $[M+H]^+$

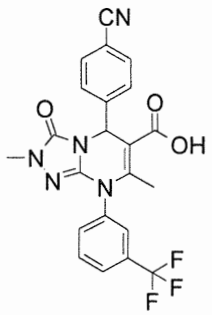
Intermediate 6



5-(4-Cyanophenyl)-2,7-dimethyl-3-oxo-8-(3-trifluoromethylphenyl)-2,3,5,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carboxylic acid allyl ester

Intermediate 5 (1.01 g, 2.88 mmol), 3-(trifluoromethyl)phenylboronic acid (1.09 g, 5.74 mmol), copper (II) acetate (525 mg, 4.27 mmol) and pyridine (455 mg, 5.76 mmol) were stirred in DCM (15 ml) under air at RT. After 16 hours triethylamine (1.16 g, 11.5 mmol) was added and the reaction was stirred for a further 3 days. Pyridine (10 ml) was then added and the reaction was heated in air at 50°C for 7 days. After cooling the pyridine was evaporated and the residue was partitioned between DCM and 1M HCl(aq). The biphasic mixture was filtered through Celite® and the organic layer was separated, dried (Na_2SO_4) and evaporated. The residue was treated with a 1:1 mixture of ethyl acetate and pentane and a solid was filtered off. The filtrate was evaporated and the crude product was purified on a RediSep® Si cartridge (80 g) eluting with 20-100% ethyl acetate in pentane. Intermediate 6 was obtained as an orange gum.
Yield: 277 mg (19%)
LC-MS (Method 1): Rt = 3.81 min, m/z = 496 $[M+H]^+$

Intermediate 7

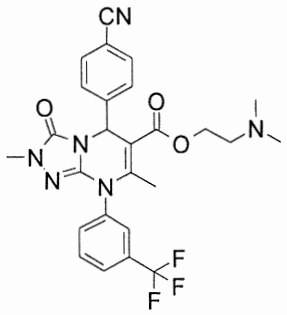


5-(4-Cyanophenyl)-2,7-dimethyl-3-oxo-8-(3-trifluoromethylphenyl)-2,3,5,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carboxylic acid

Using a method similar to that described for Intermediate 3, Intermediate 7 was prepared from Intermediate 6.

LC-MS (Method 1): Rt = 3.20 min, m/z = 456 $[M+H]^+$

Example 2

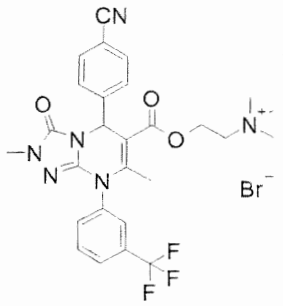


5-(4-Cyanophenyl)-2,7-dimethyl-3-oxo-8-(3-trifluoromethylphenyl)-2,3,5,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carboxylic acid 2-dimethylaminoethyl ester

Using a method similar to that used for the synthesis of Example 1, Example 2 was prepared from Intermediate 7.

LC-MS (Method 2): Rt = 3.46 min, m/z = 527.31 $[M+H]^+$

Example 3



{2-[5-(4-Cyanophenyl)-2,7-dimethyl-3-oxo-8-(3-trifluoromethylphenyl)-2,3,5,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonyloxy]ethyl}trimethylammonium bromide

Example 2 (26 mg, 0.05 mmol) was dissolved in a 30% solution of bromomethane in acetonitrile (4 ml). The reaction was allowed to stand at RT overnight. The volatiles were evaporated. The residue was dissolved in water and freeze-dried to give Example 3 as a white solid.

Yield: 31 mg (100%)

LC-MS (Method 2): Rt = 3.45 min, m/z = 541.31 $[M+H]^+$

Biological Assay

The compounds of the Examples were tested for HNE inhibitory activity.

Fluorescent peptide substrate

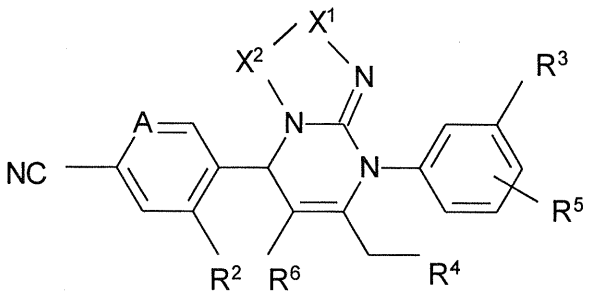
Assays were performed in 96-well plates at a total assay volume of 100µl. The final concentration of the enzyme (human leukocyte elastase, Sigma E8140) was 0.00036 units/well. A peptide substrate (MeO-Suc-Ala-Ala-Pro-ValAMC, Calbiochem #324745) was used, at the final concentration of 100µM. The final concentration of DMSO was 1% in the assay buffer (0.05M Tris.HCl, pH 7.5, 0.1M NaCl; 0.1M $CaCl_2$; 0.0005% brij-35). The enzymatic reaction was started by adding the enzyme. The enzymatic reaction was performed at RT and after 30mins stopped by adding 50µl soybean trypsin inhibitor (Sigma T-9003) at a final concentration of 50µg/well.

Fluorescence was read on the FLEXstation (Molecular Devices) using 380 nm excitation and 460 nm emission filters. The potency of the compounds was determined from a concentration series of 10 concentrations in range from 1000 nM to 0.051nM. The results are means of two independent experiments, each performed in duplicate.

The compounds had activity in the range 1-200nM.

Claims

1. A compound of formula (I):



(I)

wherein

A is C-R^1;

$\mathbf{R^1}$ and $\mathbf{R^2}$ are hydrogen;

$\mathbf{R^3}$ is 3-trifluoromethyl, 3-chloro or 3-bromo;

$\mathbf{R^4}$ is hydrogen;

$\mathbf{R^5}$ is hydrogen;

$\mathbf{R^6}$ is $-CO_2\mathbf{R^{11}}$;

$\mathbf{-X^1-X^2-}$ is $-N\mathbf{R^{19}}-CO-$;

$\mathbf{R^{11}}$ represents a radical of formula $-[Alk^1]_p-[Q]_t-[Alk^2]_q-Z$ wherein

p and **q** are independently 0 or 1;

t is 0;

Alk^1 and **Alk**^{2} each independently represent a C_1-C_6 alkylene radical;

Q represents a divalent mono- or bicyclic carbocyclic or heterocyclic radical having 3-9 ring members;

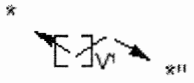
Z is

(i) -$N(R^A)(R^B)$ wherein R^A and R^B are independently hydrogen, or a C_1-C_6-alkyl; or

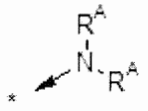
(ii) -$N^+(R^A)(R^B)(R^C)$ wherein R^A, R^B and R^C are independently a C_1-C_6-alkyl;

R^{19} is selected from hydrogen, and (C_1-C_6)alkyl.

2. A compound as claimed in claim 1 wherein R^{11} has the formula -$[Alk^1]_p$-$[Q]_t$-$[Alk^2]_q$ -Z wherein -$[Alk^1]_p$-$[Q]_t$-$[Alk^2]_q$- is structure (IV) wherein V^1 is 0, 1, 2, 3 or 4, and Z is structure (VI) wherein R^A is as defined in claim 1,



(IV)



(VI)

and wherein amine nitrogen atoms, may be quaternised with a (C_1-C_6)alkyl.

3. A compound as claimed in any of the preceding claims wherein **-X^1-X^2-** is -N**R**^{19}-CO- and R^{19} is methyl.

4. A compound as claimed in any of the preceding claims, in the form of a pharmaceutically acceptable salt.

5. A pharmaceutical composition comprising a compound as claimed in any of the preceding claims and a pharmaceutically acceptable carrier or excipient.

6. A pharmaceutical composition as claimed in claim 5 which is adapted for oral administration or administration by the pulmonary route.

7. A compound as claimed in any of claims 1 to 7 for the treatment of, or for use in the manufacture of a medicament for use in the treatment of, a disease or condition in which HNE is implicated, wherein the disease or condition is chronic obstructive pulmonary disease (COPD), chronic bronchitis, lung fibrosis,

pneumonia, acute respiratory distress syndrome (ARDS), pulmonary emphysema, smoking-induced emphysema or cystic fibrosis.